135

Vol. 57 OCTOBER, 1930 No. 7

Bulletin

of the

Torrey Botanical Club

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CONTENTS

Cicinnobolus Cesatii, a study in host-parasite relationships (plates 24-25). *Chester W. Emmons* 421

Relation of host and pathogen in the oat smut, Ustilago Avenae (plates 27-30) ..... *Laura Alma Kolk* 443

Plants observed on an excursion to Grand Isle, Louisiana ..... *Clair A. Brown* 509

Index to American Botanical Literature 515

Published for the Club monthly except
July, August, and September
by
GEORGE BANTA PUBLISHING COMPANY, MENASHA, WISCONSIN
Subscription price $5.00 per year
Entered as Second Class Matter at the Post Office at Menasha, Wisconsin

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TORREYA IS THE OFFICIAL ORGAN OF THE WILD FLOWER PRESERVATION SOCIETY OF AMERICA

TORREYA is furnished to subscribers in the United States and Canada for one dollar per annum; single copies, thirty cents. To subscribers elsewhere, twenty-five cents extra, or the equivalent thereof. Postal or express money orders and drafts or personal checks on banks are accepted in payment. Subscriptions are received only for full volumes, beginning with the January issue. Reprints will be furnished at cost prices. All subscriptions and other communications relating to the business of the club should be addressed to 450 Ahnaip Street, Menasha, Wisconsin, or to the Treasurer, Mrs. Helen M. Trelease (Mail address—Box 42, Schermerhorn Hall, Columbia University, New York City).

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Cicinnobolus Cesatii, a study in host-parasite relationships

CHESTER W. EMMONS

(WITH PLATES 24–26)

The common parasite of the powdery mildews, *Cicinnobolus Cesatii* De Bary, has been known since the appearance of the grape mildew in England and Europe near the middle of the last century. The intimate association of *Cicinnobolus* with its host makes it a favorable object for the study of host-parasite relationships. It is, at least in its earlier stages, primarily an intracellular parasite which can be followed through its development in situ. The hyphae of the mildew are large and transparent and can be successfully studied in cytological preparations and in the living condition. It is also favorable material for the study of pycnidium formation in the transformed mycelial hyphae, conidiophores, and ascocarps of its host, and in cultures on artificial media.

The earliest references to *Cicinnobolus* are descriptions of its pycnidia and spores given by students who believed that they were describing accessory spore forms of its host, *Oidium* or *Erysiphe*. The descriptions given by Amici (1853), Berkeley (1851, 1853, 1855, 1857), Figuier (1865), Montagne (1855), Graham (1851), Halstead (1888), and Cooke (1906) are of this sort. Savi (1853), Castagne (Montagne, 1855), and Crocq (1851) made the parasite the diagnostic feature of the mildew, which was then placed by each of them in a different genus. Both the Tulasnes (1861), and Von Mohl (1854) give excellent drawings of the pycnidia, but they considered them to be reproductive organs of the mildews.

Cesati (1852) recognized the fungus as distinct from grape mildew and named it *Ampelomyces quisqualis*. Riess (1852) also observed it and called it *Byssocystis textilis*, and Ehrenberg (1853) gave it the name *Cicinnobolus florentinus*. However a number of students, including the Tulasnes and Von Mohl as noted above, continued to regard it as a phase in the development of the mildew.

De Bary (1870) was the first to make a thorough study of the fungus. He rejected the names which had already been given to it as unsuitable without making his reasons for doing so very explicit, and gave the fungus the name of *Cicinnobolus Cesatii* under which it has since been known. In a few cases later investigators have used the earlier name given by Cesati. Most of De Bary's observations were on the fungus as a parasite of *Erysiphe Galeopsidis* DC. on *Galeopsis tetrahit* L. He noted that the invading hyphae

BULLETIN OF THE TORREY BOTANICAL CLUB, VOL. 57, NO. 7, OCTOBER 1930

have a diameter of about one half that of the host, that they are sparsely branched, that they grow rapidly through the host and bore through its cross walls without being constricted, and that at first the parasite seems to have little effect upon its host. He noted too that when left several hours in water the mycelium grows out from its host cell as a free filament. He observed the entrance of *Cicinnobolus* hyphae into conidiophores where they branch, and he says that these branches, by side by side approximation with each other and with the original hyphae, form a layer of cells which lines the inside of the conidiophore and encloses the central part of the host cell. He believed that this layer remains one cell in thickness. He stated that the cells which were at first hyaline and four or five times as long as broad become brownish and isodiametric. De Bary noted that pycnidia also form in mycelial hyphae and that ascocarps, too, become infected and bear the 'stylospores', as Tulasne had called them, instead of ascospores. De Bary cited ascocarps infected at various stages of development as conclusive proof that *Cicinnobolus* is a parasite and not a phase in the life of *Erysiphe*. He does not, however, figure or describe the method of entrance and spread of the parasite in the ascocarp. In the pycnidia formed in conidiophores, De Bary noted that the cells of the pycnidium wall function as 'spore-mother cells'. His figure shows the wall of the pycnidium to be composed of a single layer of cells, and it is this layer of wall cells which is sporogenous. The inner wall of a cell buds out a projection which elongates, the protoplasm passes into it, and it is abstricted as a spore. De Bary saw only one spore being formed by a cell, but because of the large number of spores contained in a pycnidium he thought it probable that spores were formed successively by the same cell. He failed to observe that the pycnidium wall may in reality be two or more cells in thickness and that the sporogenous character of these additional cell layers can account for the productiveness of a pycnidium. Before it forms a spore a wall cell is filled, according to De Bary's account, with a dense homogeneous protoplasm, afterward the contents appear watery. After the spore is shed the place where it was borne is marked by a slight unevenness. The spores are imbedded in slime and the mass of spores is exuded as a cirrhus. De Bary does not of course figure nuclei or mention nuclear phenomena. He was able to germinate the spores in water and in moist air on a leaf. By sowing spores of host and parasite together he got infection of the conidia of the mildew and of the germ tubes growing from them. The hypha of *Cicinnobolus* pierces the other, and once inside, increases in size, and then proceeds to invade other portions of its host. By cross inoculations De Bary showed that the size and shape of the pycnidium depend largely upon the host within which it grows, the organ invaded, and its stage of development at

the time. He concludes, therefore, that the forms then known probably belong to a single species.

The brief notes which have appeared since 1870 deal with the distribution of *Cicinnobolus* and describe several new species. They add little to De Bary's observations. For the sake of completeness the literature is here brought together. These students on the basis of morphological variations and host relationships have attempted to split the genus into many species, and Oudemans (1924) lists 19 species on 13 hosts. In describing a new species, *C. Humuli*, Fautrey (1890) comes to the interesting conclusion that the fruit body of *Cicinnobolus* is not a pycnidium, but a spermogonium which bears an external resemblance to a pycnidium but internally is very different.

Griffiths (1899) collected the parasite in the Northwest, and under the name of *Ampelomyces quisqualis* gives a formal description of it. In this description he reports that the fungus is sometimes found in the cells of the host plant of the mildew, although he does not mention finding it in the intercellular spaces of the leaf. In his synonymy he includes *C. florentinus* Ehr., *Byssocystis textilis* Riess, *C. Cesatii* De Bary, *C. Oidii* Tuck., *C. Humuli* Faut., *C. cotoneus* Pass., *C. Uncinulae* Faut., and *C. major* Kell. and Swingle.

Farlow and Seymour (1891, p. 174) list only the one species, *C. Cesatii* De Bary, parasitic on 'Erysipheae variae'. Seymour (1929) includes *C. Cesatii* and *C. major* on several mildews. Likewise Davis (1914) lists *Ampelomyces quisqualis* Ces. 'on various Erysiphaceae'. J. P. Anderson (1907) reports its common occurrence on a number of plants, and adds the remark that it 'undoubtedly serves to hold the mildew in check'. Other reports of its occurrence are by F. W. Anderson (1889a, b) on *Erysiphe Cichoracearum*, by Galloway (1889) on *Podosphaera Oxyacanthae*, by Kellerman and Swingle (1888), by Leunis and Frank (1886) on several mildews; and by Zeller (1929) on *Sphaerotheca Humuli*. Hino and Kato (1929), in a paper of which I have seen only the review (1929), describe a new species which they call *Cicinnobolus Asteris*.

MATERIALS

The material for the present study came from collections of *Erysiphe Cichoracearum* on *Helianthus tuberosus* growing near Ridgewood, New Jersey. This was a very abundant source, and furnished a good supply throughout the course of the study in 1928 and 1929. Of 117 collections of powdery mildews made from other sources and brought to the laboratory for microscopic examination, and of many times that number of collections examined in the field, only six were found to be infected by *Cicinnobolus*.

In one of these it was a parasite of *Uncinula circinata* on *Acer saccharinum* growing near the *Helianthus* mentioned above. The growth was very sparse. In the other five it was a parasite of *Erysiphe Cichoracearum*, twice on *Solidago bicolor*, twice on *S. caesia*, and once on *Plantago major*. These data agree with those of other students as to its sporadic occurrence. After a little practice the presence of *Cicinnobolus* can usually be detected by the naked eye. Infected patches of mildew assume a brownish color which is difficult to describe and can not always be depended upon for diagnosis, but the diagnosis can be confirmed by observing the pycnidia through a hand lens.

I have not undertaken a critical taxonomic study of the genus, but examination of collections of *Cicinnobolus* and of the exsiccati in the herbarium of the New York Botanical Garden to which I was kindly given access, lead me to believe that *C. Humuli*, *C. Uncinulae*, *C. Verbenae*, and *C. quercinus* at least should not be specifically separated from *C. Cesatii* on the basis of structural differences. Whether there are physiological species, as has been shown for the rusts and smuts, remains to be shown. There are as great variations in size and shape of spores and pycnidia within a single collection and even within a single mount from a single spot of mildew as there are between spores and pycnidia of the above named species. The shape and size of the pycnidium varies with the age and condition of the conidiophore within which it is built. The pycnidia which develop within ascocarps do not alter the shape of the ascocarp within which they are although they are regularly eccentric in their origin. Herbarium material of the collections made for this study show that the pycnidia formed in conidiophores are 25–50×50–80 microns, and that the spores are 2.5–3.5× 6.5–12 microns. The pycnidia are gold-brown to deep brown in color; they may be spherical, egg-shaped, cylindrical, or spindle-shaped; they may be sessile or stipitate; and they may or may not be crowned with a chain of immature, shrunken, and sometimes partially invaded conidia. The spores are oval, spindle- or kidney-shaped, usually inequilateral; thin-walled; and they are usually biguttulate with a rather conspicuous shining droplet at each end.

METHODS

Fresh material was studied in freehand sections and in scrapings mounted in water, NaOH, and lactophenol. Material was fixed in chrom-acetic, Bouin's, Allen's, Merkel's, and seven combinations and dilutions of Flemming's fixing solutions. Of these, Flemming's weaker and dilutions of Flemming's medium seemed to give the best fixation. The time of fixation was varied from 24 hours to 70 hours. Flemming's medium diluted with

an equal volume of distilled water and allowed to act for about 70 hours gave the finest preparations. Heidenhain's iron alum haematoxylin and Flemming's triple stains gave clearly differentiated preparations, but the triple stain was used almost exclusively.

Following good fixation the triple stain reveals nuclear and cytoplasmic structures similar to those found in other fungi. The nucleus is spherical or some times oval or egg-shaped, and is surrounded by a definite nuclear membrane. There is but little evidence of a chromatic reticulum. The chromatic material seems to be diffused throughout the resting nucleus which is of a grayish blue color. One deeply staining nucleole is present a little to one side of the middle of the nucleus. Much smaller and fainter, but showing in some of the nuclei, is what appears to be a centrosome located on the nuclear membrane. This is the usual appearance of the nucleus, but at certain stages of development or perhaps as a result of fixation the deeply stainable material is enormously increased. Not only is it more dense within the nuclear membrane, but it is aggregated on its outer surface as well, and is even dispersed through the cytoplasm. Such cells stain more deeply and are difficult to destain. In some cases it is easy to see several stages in the development of this condition in a single hypha of *Cicinnobolus*.

The cytoplasm takes the orange stain except in exceptional cases as noted above. It is finely reticulate, particularly at the rapidly growing invading tip of a hypha. A very common picture is that of a large cell with two newly formed daughter nuclei separated by a large vacuole (fig. 13). The cell wall in young hyphal tips is very thin, and it increases in thickness as the cell grows older (fig. 1, *b-j*). The wall reaches its maximum of development in certain pseudo-parenchymatous cells which form on the epidermis of the leaf or within epidermal cells of the host of the mildew (fig. 2). These will be considered later in another connection.

HOST-PARASITE RELATIONSHIPS

The cells of the invading hypha are long and narrow and the walls are thin. The cross walls are very difficult to demonstrate, and in general the walls of the parasite at this stage of development do not stain heavily. The invading hypha passes through the cross septa of the host hypha without distortion or buckling, as is shown in figure 1, *f*. This suggests that the host cells may be broken down by enzymatic action. The invading hypha passes through the wall of the host and directly into the latter's protoplasm. In my opinion there is no invagination such as we find in the invasion of epidermal cells by the haustoria of *Erysiphe*. The tip of the hypha of the parasite is surrounded by a hyaline zone (fig. 1, *b-c*). This condition is not found

in the later stages of invasion. It may be in part shrinkage due to imperfect fixation, but since it occurs only at the invading tip of the parasite it would seem to indicate a reaction on the part of the host or of the parasite. Either the metabolic activities of the parasite or shrinkage of the host protoplasm in response to the presence of the parasite might account for the condition.

The appearance of the host cell in early stages of invasion shows little or no change. This condition is in sharp contrast with that created by such a fungus as *Sclerotinia cinerea*, an intercellular parasite, which kills the tissues of its host even in advance of its hyphae. *Cicinnobolus* evidently does not form a toxin which kills the host protoplasm in advance of its growth through it. In my preparations the nucleus of *Erysiphe* contains a blue-staining chromatin, distributed in certain stages in the form of a chromatic reticulum oriented on a centrosome, and a red-staining nucleole; and it retains its size, shape, and staining reactions even after the parasite has passed through the length of the cell and into the cell beyond. The host cytoplasm is coarsely reticulate, the strands are slender and contain few granules (fig. 1, *a*). The cytoplasm of a cell which is pierced by a hypha of the parasite at first shows little change except for the presence of a hyaline zone surrounding the invading tip of the *Cicinnobolus* as described above. This hyaline zone, which seems to be evidence for an enzymatic activity of the parasite, is in turn surrounded by a zone of somewhat denser cytoplasm, in part the cytoplasm which has been displaced by the invading hypha (fig. 1, *b*). However, four or five *Erysiphe* cells back from such a cell as the one described there is distinct evidence of death and disintegration. The nuclear membrane has disappeared, and a part of the chromatin appears to have diffused out into the cytoplasm. The reticulations of the cytoplasm have become coarser and it is more granular (fig. 1, *e*). At this time there may appear to be an increase in the amount of material present in the cell, an appearance due perhaps to a change in the staining properties of the material. In the later stages of invasion the cytoplasm decreases in amount and undergoes what may perhaps be characterized as granular degeneration. The reticulations disappear and finally only a little granular material is left in the host cell (fig. 1, *g*). The changes in the nuclear material which accompany those in the cytoplasm are similar. The chromatic material is granular and ragged as one sees it in sections and it finally loses its staining reaction and disappears. The nucleole, too, is destroyed. These changes are shown in figures 1*b* to 1*g*.

The parasite itself is much changed by the time this stage of development has been reached. Whereas the cells of the young hyphal tip were long and slender and thin-walled, they have now become short and thick

by growth and septation and the walls are much thicker. Moreover, the hypha has a peculiar nodular or lobed appearance due to lateral growth of the cells (fig. 1, *g*). These lobes are in no way correlated with the branches of the host hypha; they perhaps exhibit a tendency of the parasitic hypha to branch. The lateral outgrowths from a few cells do develop into branches which then either enter into a branch of the host hypha if one is present, or grow along parallel with the first hypha (fig. 1, *h*). Only a few of these lateral lobes ever develop into branches and most of them disappear in the older hyphae when the cells become larger and the walls thicker. They may serve an important fuction by increasing the absorbing surface of the parasite during the period of their most prominent development. This period coincides with that of the cell's most rapid growth at the expense of the cytoplasm which immediately surrounds it. They may be of slight importance also in securing food for the more or less distantly developing pycnidia. The pycnidia develop most commonly in the conidiophores or ascocarps of the host. The material in a conidiophore at the time it is invaded is not sufficient for the building of a pycnidium. The host does not continue to supply food to the invaded organ, for even at an early stage of pycnidium formation, such as is shown in figure 4, the protoplasts of the host in the conidiophore and in the adjacent cells of the hypha from which it springs are nearly destroyed. Additional food must be brought into the developing pycnidium from surrounding portions of the mycelium and some of it may come from these young hyphae. Material for the later development and the maturing of the pycnidium evidently comes from the adjacent hyphae which at this stage are living saprophytically on the dead remains of the host and on other organic material present upon the surface of the leaf. This saprophytic development will be described later.

The nourishment of the developing pycnidium from a more or less distant source is analogous to that which occurs in the development of the conidiophores or ascocarps of the host, *Erysiphe*. However in the case of the mildew fungus food is secured through a specialized structure, a haustorium. The epidermal cell of the leaf tolerates the presence of the haustorium so that both remain alive. The parasite of the mildew, on the other hand, although it does not immediately kill its host, grows through it, and destroys the protoplasm in the cells through which it passes. The ability of *Cicinnobolus* to enter and grow through a cell of its host with as little disturbance as is manifested indicates a high degree of adaptability to the parasitic habit. Nevertheless it is a virulent parasite which kills the parts of its host through which it grows and then invades other portions of its host or becomes saprophytic. The question of what constitutes specialization in a parasitic fungus is well discussed by Rice (1928), who also gives a thorough review of the literature on the subject.

SAPROPHYTIC DEVELOPMENT

I have readily verified De Bary's observation that when a piece of infected mycelium of the mildew is placed in a drop of water the parasite continues to grow and in a few hours sends its branches out into the water beyond the limits of its host (fig. 12, *a*). The same thing occurs as *Cicinnobolus* grows in nature (fig. 12, *b*). The older *Cicinnobolus* hyphae are often not surrounded by remnants of the old host cell. Many of these probably grew within host cells which were later destroyed. It seems probable that others have developed as branches from hyphae of the first type and have grown out through the old host wall onto the leaf surface where they continue to grow saprophytically. The parasitism of *Erysiphe* by *Cicinnobolus* is exhibited only at the periphery of the latter's mycelial ramifications. A few host cells back from the invading tip of the parasite the protoplasts of *Erysiphe* show distinct evidence of death. Parts of the *Cicinnobolus* mycelium immediately back of these regions may get some nourishment from the younger parts; but its long continued growth and its utilization of the remains of the host protoplasm show that it can also utilize dead organic material. *Cicinnobolus* is primarily a parasite, but in point of time it is parasitic on *Erysiphe* only during the earlier period in its development.

The transition from the parasitic to the saprophytic habit is by no means clear, but it can at least be studied more objectively in *Cicinnobolus* than in the case of most pathogenic fungi. The host in most cases is a vascular plant well equipped for the transfer of food from point to point. *Albugo candida* stimulates its host to hypertrophy, so that an abnormal amount of food is supplied to the infected region. *Erysiphe Cichoracearum* does not hypertrophy its host but withdraws an abundance of food from the epidermal cells. *Pythium Debaryanum* causes necrosis of tissues and becomes saprophytic, but it is not clear when this change in habit occurs, nor to what extent the parts of the mycelium in the dead tissues of the host are nourished by the young hyphae which are advancing into living host tissue. The host presents in all these cases a more or less compact and extensive many-celled tissue. The host of *Cicinnobolus*, on the contrary, presents long hyphae through which the parasite must grow, and the changes which follow invasion and killing of the host protoplasts can be seen more clearly. They are the changes which have been described above.

Host-parasite relationship reaches its highest development in those cases where the parasite derives nourishment from its host with a minimum of injury to the latter. Whether *Cicinnobolus* is at all mutualistic at any stage is not wholly clear. It invades and kills its host, and the latter, once it is invaded, shows no obvious protective reaction. It is liable to invasion

at almost any stage of development. Only the mature conidia and the nearly mature ascocarps are, apparently, secure against the parasite. One finds ascocarps of nearly all stages up to maturity invaded by the parasite. Its virulence is shown by its complete invasion of all the mycelial ramifications of its host and the resultant transformation of the fruiting structures of the latter into its own pycnidia. It is specialized in its choice of host, in its method of invasion, and in the completeness of its utilization of the materials in its host.

PYCNIDIUM FORMATION

Pycnidia develop in conidiophores in the following fashion. When a hypha of *Cicinnobolus* which is growing inside a hypha of *Erysiphe* reaches a conidiophore it sends one or more side branches into it. If two branches enter the conidiophore they may be from adjacent cells (fig. 3), or they may be two which have arisen farther back when the hypha of the parasite branched within that of its host (fig. 6). Whether the conidiophore is entered by branches arising immediately at its base, or by branches of more distant origin, or by a single branch (fig. 4) makes no essential difference in the subsequent development. The formation of the pycnidium is initiated by the branching of the hypha or hyphae which have entered the conidiophore of the host. The fact that the branching may sometimes begin some microns from the base of the pycnidium does not alter the type of development, which is symphyogenetic, as noted by De Bary (1884,1887).

When a conidiophore of the host is entered by a single hypha of the parasite the latter grows the length of the conidiophore, piercing if they are present one or more of the septa of the host. Upon reaching the distal end of a young conidiophore (fig. 6), or the thick wall which forms the base of a nearly mature conidium of the host (fig. 5) the parasitic hypha may turn back and grow again toward the base. In the meantime several of the cells of the invading hypha have formed lateral branches which leave it at nearly right angles (fig. 4). De Bary says that the branches which begin the formation of the pycnidium grow upward so as to make an acute angle with the hypha from which they originate, and that they retain their identity for a rather long time. I find that the first branches in an invaded conidophore leave the main hypha at about right angles. The cells of the parasite soon become isodiametric and grow in size and divide radially as well as transversely, so that the original hyphae soon lose their identity (fig. 6). Growth and radial division nearly fill the infected conidiophore although there seems to be a lumen always present at the center. This is in harmony with De Bary's description of a central cavity left in the host cell by the parasite which lines its inner wall. In comparing infected and

uninfected conidiophores I see no evidence of hypertrophy at this stage of development. A stretching of the host wall occurs at about this time, but I interpret it as a passive stretching caused by the growth of the parasite. By the time a pycnidium has reached the stage of development shown in figure 6 the nucleus and cytoplasm of the cells have already been destroyed. Moreover the host protoplasts in the hypha which bears the conidiophore have previously been killed, and the cells of the infected conidiophore are in no condition to respond to a stimulus to hypertrophy if such a stimulus were present. I have not seen hypertrophy in invaded mycelial hyphae. The size of invaded ascocarps as we shall see later seems to be slightly less than that of normal ones. The effect of *Cicinnobolus* upon *Erysiphe* is necrotic.

Growth and radial division now increase the diameter of the young pycnidium. Its wall may become two or three cells thick, probably by tangential divisions (fig. 8–10), but the cavity at the center does not become filled. From this it is evident that in its development the pycnidium of *Cicinnobolus* does not conform absolutely to either the symphyogenetic or the meristogenetic type of formation. In its early stages it is of the former, and in its later stages it more nearly resembles the latter type. The outside layer of cells forms the outer surface of the pycnidium; it does not appear except at the base and at the summit to be enclosed in the old conidiophore wall. It is differentiated from the deeper layers only by the slightly heavier walls of its cells, and all layers are sporogenous. These conditions are in disagreement with the conceptions of De Bary, who figures a portion of a pycnidium wall composed of a single layer of cells.

The spores of *Cicinnobolus* are formed in a manner unlike that known in other fungi. A swelling appears on the inner wall of a cell which borders on the central cavity (fig. 8). This swelling gives rise to a spherical or elongated outgrowth from the cell, which is the young spore (fig. 8). The wall of this young spore appears to be continuous with that of the cell from which it arises. The young spore acquires almost the volume of the cell which formed it, and into it pass the nucleus and nearly all the cytoplasm of its mother cell. The spore then breaks loose and assumes the size and shape characteristic of *Cicinnobolus* spores. The spores are uninucleate and thin-walled. When cell division has immediately preceded spore formation the two daughter cells may be separated by a very thin wall, barely visible. In such cases it at first appears that there is a single large binucleate cell (fig. 9). Closer examination usually reveals a wall separating the two protoplasts. Such cells as are shown in figures 9 and 10 represent exceptional rather than typical cases. It is possible that sometimes this wall does not develop, in which case one cell would produce two spores.

Spore formation in *Cicinnobolus* as here described bears a superficial resemblance to that which Adams (1920) figures for *Darluca filum*. However, there is this important difference, a sporogenous cell of *Cicinnobolus Cesatii* bears a single spore, while the figures which Adams gives for *Darluca* show that a nucleus remains in the cell after the formation of the spore. No description of spore formation is given in the text of the article. The sporogenous cells of *Cicinnobolus* at certain stages resemble in slight degree those of *Mycosphaerella Bolleana* and of *M. personata* which, according to Higgins (1920, 1929), give rise to spermatia. However, the sporogenous cells in *Cicinnobolus*, so far as I have found, give rise to a single spore each. Jenkins (1930) reports that spermogonia similar to those described by Higgins are also present in *M. cerasella*, and that spermatia are formed in a similar manner, each fertile cell producing three or four. He further reports that spermatium formation proceeds centrifugally as the spermogonium develops. He thinks that this fact may possibly be of phylogenetic interest. In *Cicinnobolus* also spore formation proceeds centrifugally.

After spore formation has been completed the walls of the empty cell gelatinize (fig. 10) and contribute to the slimy substance which holds the discharged spores together in the cirrhus which is well developed in *Cicinnobolus*. The cells underneath now in turn become sporogenous. All cells including those forming the external wall of the pycnidium are potentially sporogenous (figs. 8–10). The cells toward the periphery of the pycnidium differ from those toward the middle by having thicker walls which do not wholly gelatinize (fig. 8), but they form their spores in the same way (fig. 8). Spore formation is completed first near the top of the pycnidium and many sections show the empty cell walls of exhausted cells at the top of the pycnidium while there are still many spore-bearing cells toward the base (fig. 9). Indeed, the cells at the base of the pycnidium may not produce spores during the summer or fall, but are found alive and in good condition late into the winter. Except for these cells whose spore production seems to be only delayed, all the cells of a pycnidium including those forming its external wall produce spores. No ostiole is formed and the spores escape through an irregular aperture near the apex of the pycnidium where the empty cell walls break. If two hyphae instead of one enter a conidiophore they do not usually turn and grow back toward the base. Otherwise development proceeds as described above.

A pycnidium arising in an ascocarp develops in a different way. In *Erysiphe Cichoracearum* infection of the ascocarp may take place at any stage of development before the time of ascospore formation. Even the antheridia and oogonia may be attacked and destroyed (fig. 15). These structures as well as very young ascocarps are invaded when the hyphae

upon which they are borne are parasitized, but they never become the sites of pycnidia. If, however, the ascocarp is forty-five to fifty microns in diameter or larger, with its perithecial envelope showing some differentiation, it may be transformed into a pycnidium. I have never seen penetration of the perithecium by a germ tube from a *Cicinnobolus* spore, and believe that initial infection always takes place in the thin-walled mycelial hyphae of the mildew. The advance is apparently from the mycelium into the ascocarp through the ascogone or antheridial branch. Inside the ascocarp the parasite spreads from cell to cell and invades all parts of the structure (fig. 18). In size, infected ascocarps seem somewhat smaller than uninfected ones, which appear to be of about the same stage of development. It seems probable that this difference in size is due not so much to the presence of the parasite in the ascocarp itself as to the dwarfing effect of its presence in the mycelium which nourished the young fruiting structure of the host. In shape, the infected ascocarp is unchanged, and the thick walls of the outer perithecial cells and those of the appendages are never destroyed. The latter however enclose not the protoplasts of the mildew but the cells of the parasite. In later stages all remnants of the cell walls of the host in the central portions of the ascocarp disappear and the cells of the parasite are closely packed together, but in the early stages the branching hyphae of the parasite are very loosely knit and are of about uniform distribution throughout the ascocarp (fig. 18).

The pycnidium itself now begins to form near the periphery and toward the distal portion of the ascocarp in a region differentiated by the shape and staining reactions of the cells and by their relations to each other (fig. 19). The differentiation is initiated by an increase in the rates of growth and of cell division. The parasitic hyphae become densely interwoven and no obvious intercellular spaces remain. The cells which were typical hyphal cells divide and grow until they are essentially isodiametric. They stain more deeply than cells in the undifferentiated regions. Growth of the pycnidium proceeds radially from the original point of differentiation by the division of cells at its surface and by the incorporation with them into a pseudo-parenchymatous mass of the hyphae which are encountered during this radial growth of the structure. When the pycnidium reaches the outer perithecial cells toward the distal portion of the ascocarp its growth is checked in that direction but it continues conewise toward the base (fig. 21).

Cavity formation begins soon after the pycnidium is twenty-five microns in diameter, and it is of the lysigenetic type. A few cells at the center of the pycnidium degenerate, and this region of degeneration spreads by the involvement of neighboring cells until it is three or four cells in di-

ameter. The degenerating cells resemble those Dodge (1923) has described for *Phyllosticta carpogena*. The stages in the degeneration of the cytoplasm are shown in figure 17; and the development of the cavity is shown in figures 20 and 21. It will be seen that *Cicinnobolus* is a particularly good fungus to illustrate the lysigenetic formation of the pycnidial cavity. The situation is not complicated here by the presence of an upward growing layer of hyphae which exert pressure. If pressure is present at the center of this pycnidium it would appear that it is only the mutual pressure of the cells in the center of the mass upon each other. The mass of cells at this time resembles a parenchyma, and there is no apparent orientation of cells (nor of hyphae, since the original hyphae have wholly lost their identity) toward the center or the top of the young pycnidium. Possibly some diffusible substance from the cells which make up the pycnidium becomes concentrated at this point in sufficient quantity to cause the destruction of the cells. It may be the same substance which later on and in reduced concentration enables the sporogenous cell to resume growth at a certain point on its surface—a growth which eventually results in the peculiar type of spore formation described in an earlier paragraph.

As soon as the cavity is formed spore production begins. Spores are formed exactly as they are in a pycnidium formed in a conidiophore; cells which abut on the central cavity bud out into it, and the bud becomes a spore after the protoplasm of the cell has passed into it (fig. 16). Most of the wall now disappears and probably goes to help make up the gelatinous mass in which the exuded spores are imbedded (fig. 22). The cells underneath now in turn produce spores, and the method of spore formation is always the same. There are no conidiophores but the protoplasm of each cell passes directly into the growing spore. Spore production proceeds in this manner to the surface of the pycnidium. Figure 23 shows a late stage in the development of a pycnidium filled with spores which has grown nearly to the outside of the infected ascocarp.

Cicinnobolus pycnidia which form in conidiophores and those which form in ascocarps differ in the degree to which their size and form are determined by the host structures in which they develop. Pycnidia formed in conidiophores are typically flask-shaped, but the shape may be modified in a number of ways. They may be sessile or stipitate, they may be nearly spherical or cylindrical, and they may or may not be crowned with a chain of dead and shrunken cells of the host. This crown occurs only when an old conidiophore bearing nearly matured spores is invaded. The invading hypha of the parasite often enters these cells of the host, or at least the lower ones; but it does not proliferate in them and the pycnidium proper is built in the lower portions of the conidiophore. If a young conidiophore

is invaded the resulting pycnidium is rounded and smooth on its top. The age and condition of the conidiophore influence the shape of the pycnidium, and to this extent the structure of the latter is determined by the host. However at maturity it may be at its widest part several times the diameter of the original conidiophore. The mature fruit body seems to be a definite heritable structure despite the many modifications it may assume under the conditions imposed by the host.

The pycnidium is formed in a very different manner in an ascocarp. If the ascocarp be too young or too old no pycnidium is formed; but if it be of a suitable size and age the structure is soon filled with a tangled mass of *Cicinnobolus* hyphae. These invade nearly every cell of the host, entering even the cells of the perithecium and the appendages (figs. 19 and 21), but they do not at first fill all the space. They grow at random in a region limited only by the perithecium of the host. I have already traced the changes which take place here and have shown that eventually nearly the entire ascocarp is at one time or another densely packed with cells which are potentially sporogenous. In contrast with the other type of pycnidium, the only manifestation of heritable factors for form is the initiation of the pycnidium in the distal portion of the ascocarp, and its growth toward the base. The parasite never at any time modifies by its presence in the structure, either its size or shape, as is shown by the external appearance and by sections of infected ascocarps. Measurements of normal and of infected ascocarps which appear to be of about the same stage of development indicate that the latter may be somewhat dwarfed. The obvious explanation for this condition is that the presence of the parasite in the mycelium decreases the amount of food available for ascocarp formation and in consequence the latter remains smaller. So long as the parasitic mycelium remains loosely interwoven in the infected ascocarp, that is, until the pycnidium begins to form, the stage of development of the ascocarp at the time it was invaded can usually be estimated with considerable certainty. One can usually see the old cell walls of the host and can tell whether the invaded cells were nurse tussue or asci (fig. 19). Infected ascocarps are identified solely by the presence of the cells of the parasite in place of the protoplasts of the host. Many of the cell walls of the perithecium remain to a late stage, and they as well as those in the center of the ascocarp can be seen to contain two or more cells of the parasite (fig. 18).

DEVELOPMENT IN CULTURE

The spores of *Cicinnobolus* germinate readily in distilled water or on agar. The germ tube is of about the thickness of the spore and it may

arise from either end, or germination may be from both ends (fig. 11). The newly formed spore contains typically two conspicuous guttules, one at each end. During germination these droplets break up into smaller bodies which are diffused through the spore and the germ tube and finally disappear. The germ tube branches and soon becomes septate. Some spores, as De Bary also observed, become septate before germ tubes are formed. When spores are transferred to agar the development of the fungus is rather slow, but at the end of four or five days growth becomes evident. On dextrose, maltose, glucose, and honey agar the growth is high and compact with a wrinkled surface and little free aerial mycelium. Microscopic examination shows many swollen vesicular cells. There are few pycnidia, but some spores are formed. The color is salmon to ochraceous salmon, as determined by comparison with Ridgway's *Color Standards and Color Nomenclature*. Gelatin is liquefied. On Czapek's agar the growth is restricted to a narrow region along the line of inoculation. The mycelium is mostly submerged or at the surface. The color is pale salmon. On corn meal agar the growth seems to be more nearly normal with more aerial mycelium, but here too the fungus never covers the whole surface of the slant. The color is olive-buff to deep olive-buff and the spore masses are avellaneous.

When the colony is a week or a few weeks old pycnidia become visible as honey-colored to brown spots against the gray background of the mycelium. Later these may appear on the surface of the agar where there is such a sparse development of mycelium that it is hardly visible to the eye. The pycnidia in the cultures first isolated from infected leaves were merely loose masses of hyphae, and studies of fresh mounts in water or hydroxide and of paraffin sections revealed little of definite structure. A loose mass of large vacuolated cells was surrounded usually by a single layer of cells differentiated to form the pycnidium wall. The cells making up the pycnidium were isodiametric and showed no orientation toward the center of the pycnidium. Cavity formation was of the lysigenetic type. Spores were formed as in other types of *Cicinnobolus* pycnidia, and were finally discharged in liquid mounts in a more or less perfect cirrhus.

It may be that this imperfect pycnidium formation was the result of bacterial contamination. Later isolations of the fungus have given cultures which resemble those first secured, except that the pycnidia, particularly those which develop rather late in the culture and outside the region of most vigorous mycelial growth, are definite flask-shaped structures with a definite beak and ostiole. Besides the strain used in this study, I have also examined material which Dr. E. M. Gilbert was kind enough to send me from Wisconsin. Some fifteen monospore cultures of each of these two strains have been kept and observed for several months. The strain from

Wisconsin is darker colored, producing an olivaceous mycelium on corn-meal agar and an olive pigment which diffuses to some extent into the medium. This is a character which sometimes appears in cultures of the New Jersey material but not constantly as in the former. Both strains produce pycnidia which are flask-shaped, each with a more or less pronounced beak from which the spores are exuded through an ostiole as a cirrhus. The pycnidia measure about 50–80×100–170 microns. Those produced by the Wisconsin strain are somewhat smaller than the others. The spores measure 2.5–3×6–9 microns, and they appear to be formed in the same way as in the pycnidia developing in the host. In its development the pycnidium exhibits the symphyogenetic type and it may be either simple or compound. One or a few hyphae branch and intertwine, and this knot of hyphae increases in size by growth of the hyphae which are already present and by the addition of other hyphae at its surface. The stages in development are similar to those described by Kempton (1919). The two strains in culture showed no mating reaction when grown together.

The winter condition of *Cicinnobolus* has so far not been described. Material collected during November and later shows that it may develop further as a saprophyte on the leaf of the host plant of the mildew, in this case *Helianthus tuberosus*. It enters the epidermal cells and the intercellular spaces of the leaf of the Jerusalum artichoke in the following manner: the hyphae apparently do not pass directly from the hyphae of the mildew into the epidermal cells of the leaf, but they have an intermediate saprophytic stage, a condition noted above.

Certain of the branches of the mycelium which are growing on the surface of the leaf now appear to reach a degree of maturity and grow and divide in such a way as to form small cushion-like masses of rather thick-walled isodiametric cells (fig. 2). These masses of cells are closely appressed to the surface of the leaf, and it is from them that hyphae grow which pierce the epidermis and give rise to ascocarps. It may have been such structures which De Bary saw and likened to *Coleochaete*. The structural relations seem to show very clearly that these saprophytic hyphae develop from the *Cicinnobolus*. I have not yet however completed the proof by infecting the mildew from them. Penetration of the wall of an epidermal cell is usually underneath one of these pads, although in some cases there is only a very rudimentary development of such a structure. In any case the epidermal wall is broken down and the hyphae enter the cell (fig. 2). Penetration is not by means of a small penetration tube through a small pore as in the case of the mildew. It is rather a mass invasion of nearly or quite dead tissue, for the leaf at this stage is nearly ready to fall. The hyphae

grow and soon fill the epidermal cell with cells resembling those which formed upon the surface of the leaf. Sometimes one or more end or side walls are broken down and the fungus spreads to neighboring cells. Sooner or later it breaks through the inner wall of the epidermal cell and enters the intercellular spaces of the leaf. I have never seen the parasite at this stage in the palisade or mesophyll cells of the leaf. Invasion of the intercellular spaces of the leaf is not always through the epidermal cells as just described, but the hyphae from the leaf surface sometimes pass through the stomata. In the intercellular spaces the type of growth is quite different. Instead of forming thick-walled nearly isodiametric cells resembling resting cells as it did within the epidermal cell the fungus now reverts to the hyphal habit of growth (fig. 2). The hyphae retain the blue stain well and are conspicuously differentiated in the leaf tissues. Their further development into what is apparently the ascigerous stage of *Cicinnobolus* will be described in a future paper.

SUMMARY

Since the time of De Bary, *Cicinnobolus* has been a familiar and classic example of a fungus living parasitically upon another parasite, the economically important mildew. It grows as a virulent parasite within the hyphae of its host, *Erysiphe*, killing the latter and utilizing almost completely its protoplasm. It then becomes saprophytic upon the surface of the leaf of the host of the mildew, *Helianthus tuberosus* in this case, and finally enters the epidermal cells and then the intercellular spaces of the latter. It forms pycnidia by the transformation of the mycelial hyphae, the conidiophores, and the ascocarps of its host into its own fruiting structures. Its host-parasite relationships, its pycnidium and spore formation during its parasitic life, and its change from the parasitic to the saprophytic habit are here described. The development of the perfect form of the fungus is to be followed in a future paper.

It is a pleasure to acknowledge my indebtedness to Professor R. A. Harper, who suggested this problem, for his generous and constructive criticism and his guidance during the study.

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Explanation of plates 24–26

Plate 24

Fig. 1. Stages in the penetration of the host hyphae: *a*, normal uninfected hypha, ×1200; *b*, *c*, tips of invading hyphae, ×2200; *d–g*, stages in the disintegration of the host cytoplasm ×1200; *h–i*, older *Cicinnobolus* cells in the hyphae of the host; the host cytoplasm has been nearly destroyed, ×2200; *j*, hypha of the parasite within a hyphal appendage of an infected ascocarp of the host; three of the nuclei are in stages of mitosis, ×2200.

Fig. 2. Pad of pseudoparenchymatous cells upon the leaf surface. The pad developed between the cutin and the epidermal cell walls. The parasite has penetrated into the epidermal cells and a pair of young hyphae have entered the intercellular spaces of the leaf. ×810.

Fig. 3. Two hyphae which have arisen as lateral branches from adjacent cells of the parasite and have entered the base of a conidiophore. ×2200.

Fig. 4. The upper part of a conidiophore invaded by a single hypha of the parasite. The parasite has pierced one septum of the host but was turned back at the one above and has begun to branch. ×2200.

Fig. 5. A later stage in invasion of a conidiophore showing how the invading hypha grows back toward the base. ×810.

Fig. 6. Invasion of a conidiophore by two hyphae which arose by branching within the mycelial hypha of the host. ×810.

Fig. 7. Cross section of a young pycnidium of a stage a little later than is shown in figure 6. ×2200.

Plate 25

Fig. 8. Section through a young pycnidium showing eight spores and several wall cells in various stages of spore formation. ×1200.

Fig. 9. Section of a small pycnidium showing one nearly mature spore and several sporogenous cells toward the base and the empty walls of exhausted cells toward the top. ×2200.

Fig. 10. Similar to figure 9. ×2200.

Fig. 11. Germinating spores. ×1200.

Fig. 12. *a*. Growth of the hypha of *Cicinnobolus* outside the host cell of *Erysiphe* after being left in water two hours. ×1200. *b*. From a stained section showing *Cicinnobolus* growing out through the wall of the host.

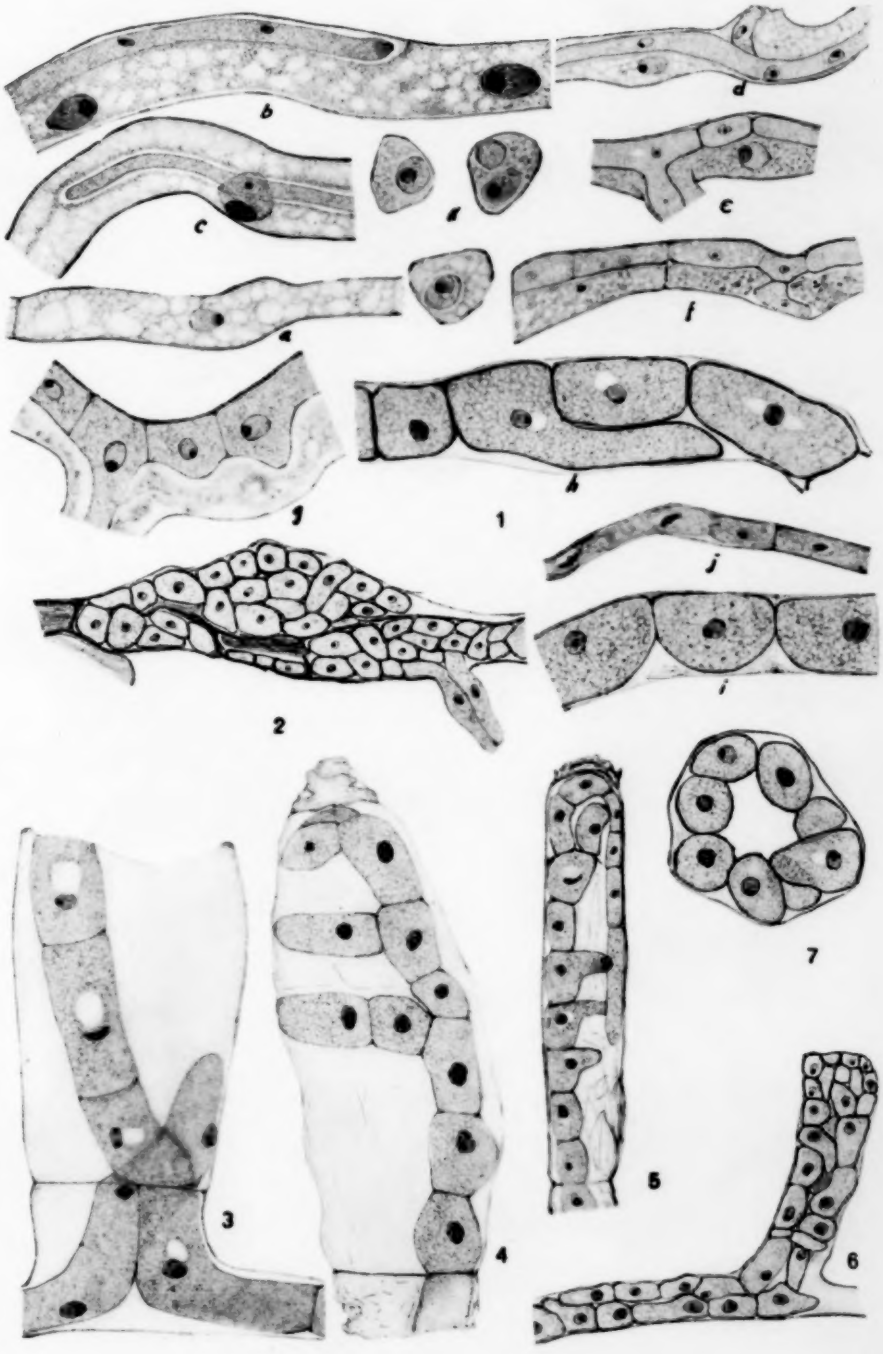
Fig. 13. A cell of *Cicinnobolus* in which the nucleus has just divided. ×2200.

Fig. 14. A cell of *Cicinnobolus* in which the appearance of the nucleus suggests a telophase. ×2200.

Fig. 15. An antherid and an oogone of the host invaded by *Cicinnobolus*. ×2200.

Fig. 16. Stages in spore formation in a pycnidium formed in an ascocarp. ×2200.

Fig. 17. Lysigenetic cavity formation at the center of a pycnidium in a transformed ascocarp. These cells are at the center of the ascocarp in figure 20. ×2200.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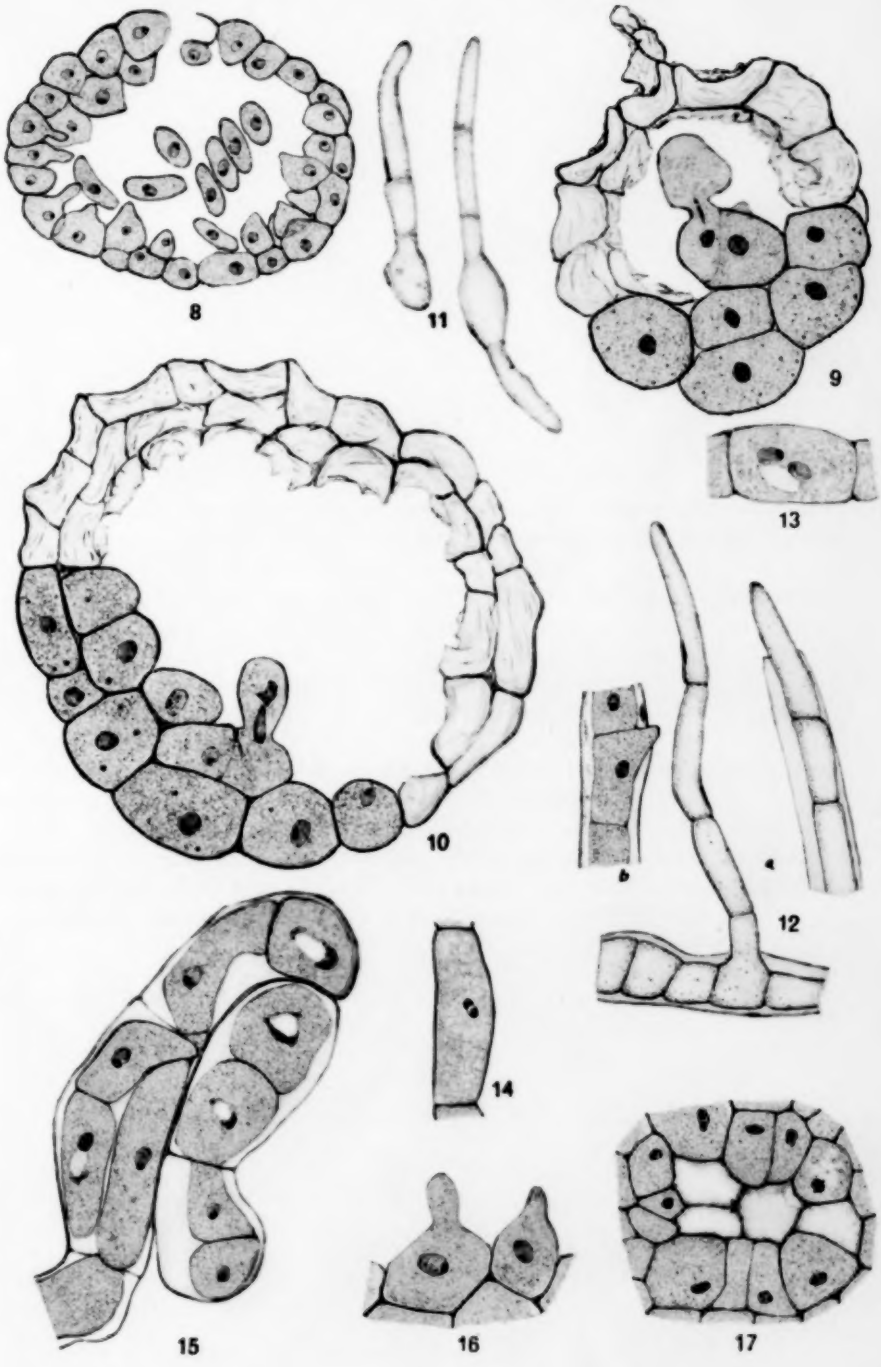
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PLATE 26

All magnifications the same and about 675 diameters.

Fig. 18. An ascocarp of *Erysiphe* completely invaded by *Cicinnobolus*. The pycnidium has not begun to form. All the protoplasts seen are those of the parasite, but the old host walls can be seen in many places.

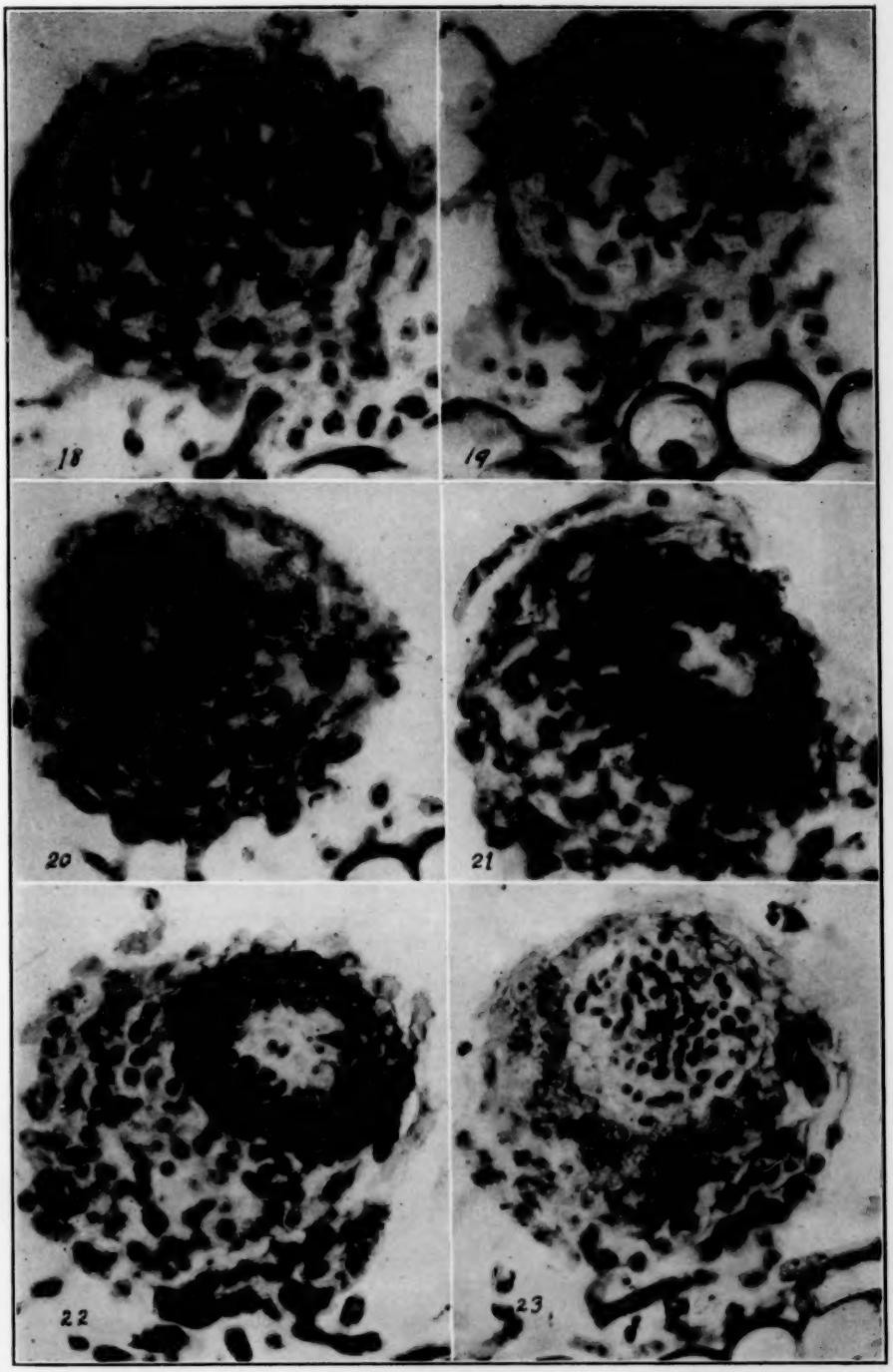
Fig. 19. An invaded ascocarp in which the pycnidium is beginning to form in the distal part. Appendages of the ascocarp containing the cells of the parasite can be seen.

Fig. 20. A parasitized ascocarp in which the pycnidium is well formed. A cavity formed by the disintegration of the cells at the center is apparent.

Fig. 21. A pycnidium in which the cavity has been fully formed. A few spores have been discharged into the cavity.

Fig. 22. A pycnidium in which a few cells can be seen in stages of spore production. Several spores lie in the cavity at the center of the pycnidium.

Fig. 23. A pycnidium which has greatly increased in size by peripheral growth. The cavity is filled with very numerous spores. The empty cell walls seen next the central cavity are those of the host. The cells of the parasite which were within these cells have produced spores as progressive cell layers have become sporogenous.



EMMONS: CICINNOBOLUS CESATII

Relation of host and pathogen in the oat smut, Ustilago Avenae

LAURA ALMA KOLK

(WITH TWENTY-ONE TEXT FIGURES AND PLATES 27–30)

CONTENTS

INTRODUCTION 443
REVIEW OF LITERATURE 444
Introductory 444
Place of penetration of the fungus into the host plant 445
Seedling infection 445
Flower infection 447
Method of penetration of the fungus into the host plant 451
Cytological evidence as to the relation of host and parasite 453
Physiologic forms and heterothallism in connection with the host-parasite relation 456
RESULTS OF ORIGINAL INVESTIGATIONS 462
Material and methods 462
Anatomy of the young seedling 464
Percentage of smutted plants and microscopic examination of seedlings 465
Place of entrance of the fungus into the seedling and distribution of the mycelium 466
Cytological study of the relations of host and pathogen 474
Initial penetration 474
Penetration from cell to cell 477
Course of the hyphae through the cells 479
DISCUSSION 480
SUMMARY 498
LITERATURE CITED 499
EXPLANATION OF PLATES 506

The recent increase in our knowledge of the smut fungi is impressive. The existence of biological strains of barley, oat, wheat, and corn smuts is well established. The existence of heterothallism is suggested in a considerable series of forms. Flower infection, it is claimed, is present in certain forms of oat smuts, as well as in loose smut of wheat, barley, and rye.

Nevertheless the old problems as to the exact place on the seedling at which infection occurs, and the normal path of the hyphae in reaching the growing point remain uncertain. The point of origin of the binucleate cells and the method of their propagation are still to be determined. We need to know much more as to the inter- and intracellular host-parasite relations such as method of penetration of host cell wall, course of the hypha through the cytoplasm of the host cell, and reaction of the host cell to the presence of the pathogen.

Recent investigations, both in Germany by Zade and his students, and in the United States by Gage, raise doubts as to whether seedling infection is the method by which *Ustilago Avenae* and also *Ustilago levis*, the loose and covered smuts of oats, respectively, gain entrance into the host plant. A new type of flower infection, differing from the well known flower infection of wheat and barley, where the smut gains entrance to the interior of the ovary through the stigma and style, is described by these investigators. In this new type, the smut instead of penetrating directly to the embryo 'infests' principally the glumes and the epidermis of the ovary at the time of flowering, and later infects the young seedling developing from such kernels. If these claims are true, the validity of tests hitherto made for varietal resistance and susceptibility of oats to loose and covered smut (Reed, 1925a), and also tests of disinfectants used in seed treatment (Tisdale, Taylor, Leukel, and Griffiths, 1925; Leukel, 1926), where the method of dry spore inoculation has been practiced, are brought in question. Little is known about the actual penetration of the mycelium into the seedling. There may be little difference between the method of seedling infection by freshly germinating spores and by resting mycelium. It is in the seedling that the smut becomes an active parasite, and the relation of host and parasite can be observed. The presence of the mycelium in the glumes cannot be considered an actual invasion of the host, unless we have some evidence that the growing point of the embryo is reached by this mycelium before the seed matures, or at least that the mycelium can be demonstrated in other parts than the protective coverings. No such evidence has yet been presented.

I have endeavored by a study of fresh material, as well as material fixed and embedded in paraffin, and then sectioned by the microtome, to obtain cytological data on some of these problems—especially how the loose smut of oats gets into the oat seedling, the distribution and cytology of the mycelium in the young seedling, and the relation of host and pathogen.

REVIEW OF THE LITERATURE

Although Zade and his students and Gage have reviewed the literature on seedling and flower infection by smuts, they have neglected to emphasize that even the early investigators of flower infection recognized that the form of the old *Ustilago Carbo* Tulasne on oats, now called *Ustilago Avenae* (Pers.) Jens., did not fit perfectly into the category either of a seedling infecting or flower infecting smut. Brefeld and Falck (1905) and Falck (1908), whose work will be reviewed later in more detail, were well aware that flower infection was possible. It is now accepted that true flower

infecting smuts, such as the loose smut of wheat and the loose smut of barley, produce spores which do not form conidia, and which are carried at flowering time to the stigmas of the flowers where they germinate immediately and penetrate the tissues of the stigmas and style down to the ovary. Seedling infecting smuts produce spores which usually become attached to the hairy pericarp of the kernels when the smutted panicles are mingled with the sound heads. They produce conidia, usually the following spring, which germinate and penetrate the tissue of the young seedling. *U. Avenae* is like the flower infecting smuts in the appearance of the smutted panicles and the dissemination of its spores at flowering time. The spores are assumed to lodge between the ovary and the glumes of the flower. It is like the seedling infecting smuts in its production of conidia on germination of the spore, and up until the present at least, it has been considered a seedling infecting smut in its method of gaining entrance into the host plant. The new idea advanced by the above mentioned investigators is that glumes and pericarp become infested with dormant mycelium of *U. Avenae* produced by conidia from spores lodged in the flower at blossoming time, and this mycelium, unlike that of the true flower infecting smuts, which immediately makes its way to the embryo, does not invade the embryo until the seed germinates. Inasmuch as Zade and his students and Gage have overlooked especially the work of Falck in raising this question as to the place of entrance of *U. Avenae* into the oat plant, it seems desirable to review again, even at the risk of repetition, the literature on this subject of the penetration of host plants by smut fungi.

Place of penetration of the fungus into the host plant

Seedling infection. The root or the root node was regarded by some of the early workers on smuts as the place of entry of the parasite into the host plant. Kühn (1859), who was the first to figure smut mycelium in the host plant, never found mycelium in the root tips of the young wheat plant infected with *Tilletia Caries*, but always found it in the region of the root node; Hoffman (1866) figured the mycelium of *Ustilago Carbo* (Flugbrand) in the young root of *Hordeum distichum* and also in the root hairs. In *Secale cereale* he gave inconclusive evidence for entrance through stomata of the coleoptile. Neither of these writers observed actual entrance of the fungus into the seedling.

Later, in his well-known controversy with Wolff, Kühn (1874) maintained that infection was assured only when penetration took place in the portion of the seedling lying between the root node and the coleoptile node. He admitted that penetration through the coleoptile did take place, but believed that infection in this way was uncertain, whereas Wolff (1873)

maintained that penetration normally takes place through the coleoptile. Wolff described the germ tube in the act of penetrating the cuticle and epidermis of this organ. Kühn's evidence *against* the significance of penetration through the coleoptile, was based on his finding masses of germ tubes in the tissue of the coleoptile of barley plants, which on maturing bore sound heads. He also observed this in plants of *Bromus secalinus* and *B. mollis* inoculated with *Ustilago bromivora*. On the other hand, Wolff's evidence *for* the possibility of penetration through the coleoptile was obtained by inoculating rye seedlings by placing germinating spores of *Urocystis occulta* on the coleoptile, examining a bit of the epidermis for the presence of the fungus, and then growing the plants either in field soil or nutrient solution until the appearance of the smut (8 or 9 weeks). He never observed penetration at the root node. To substantiate his contention that the mycelium grew straight across and through the coleoptile to the first leaf, and thence into the closely packed plant parts, Wolff gives a figure of hyphae going across the space between the coleoptile and the first leaf (pl. 8, fig. 18).

Brefeld (1895) from his study especially of *Ustilago Carbo* on oats, claimed penetration took place in all parts of the young seedling from the root node upward to the tip of the coleoptile, in the young roots, and also in the root hairs. This author's extensive and invaluable contributions to the life histories of the different smuts (1883, 1895, 1905) consisted in studies of their germination in culture media, and their methods of infection. He performed inoculation experiments with *Ustilago Carbo*, on oats and barley, *U. cruenta* on sorghum, and *U. Maydis* on corn by spraying the plants in various stages of development with conidia produced in nutrient solutions by germinating spores. His experiments on flower infection will be referred to later. Brefeld described characteristic holes in the cuticle of the coleoptile of three day old oat seedlings, caused by the penetration of the fungus. He confirmed Wolff's observation of the crossing over of the fungus hypha from the coleoptile but only in the lower part of the coleoptile.

Although Clinton (1897) did not observe actual penetration of the epidermis in his study of the smut on broom corn, *Cintractia Sorghi-vulgaris* (now *Sphacelotheca Sorghi*), he gives evidence on the distribution of the mycelium. Mycelium was always most abundant in the region where the epicotyl and the leaf sheath joined, and very little if any, was found toward the seed end of the epicotyl.

Among the more recent investigators, the coleoptile is mentioned most frequently as the place of entrance of the smut fungus into the host plant. Lutman (1910) obtained infection of oat seedlings with *Ustilago levis* by smearing agar cultures of conidia on the coleoptile.

Lang (1912), however, considered the mesocotyl (Keimknotenstück) as the point of entry. In loose smut of oats, he claimed to have found penetration, not in the earliest stages of germination, but somewhat later, in the region between the root node and the coleoptile node, and then only in cells which presented a sharp contrast in color to healthy cells. He believes that an actual penetration and growth through the host cells can take place only when degeneration of the latter has begun. Unfortunately he gives no drawings to confirm his statement as to the degenerate condition of the penetrated host cells.

Dastur (1921) and Sartoris (1924) claim that the infecting hyphae of *Tilletia Tritici* enter the wheat seedling between the epidermal cells of the coleoptile. Woolman (1923) in a preliminary report on this same smut, and Noble (1924) studying *Urocystis Tritici*, noted penetration through the epidermal cells of the coleoptile in both resistant and susceptible plants. In his completed study Woolman (1930), is of the opinion that the hypha of the bunt fungus penetrates between the epidermal cells and then enters the lumen of an adjacent cell. It is thus in the first phase of infection both inter- and intracellular and is Gram-negative in its staining reactions. As it progresses deeper in the coleoptile (second phase) it is still both inter- and intracellular, but is Gram-positive, and finally as it makes its way into the nodes, internodes, and growing point (third phase) it is strictly intercellular.

In the onion smut, *Urocystis Cepulae*, Anderson (1921) and Blizzard (1926) maintain that infection takes place through the cotyledon (the base of the cotyledon serving as a sheath through which the first leaf emerges).

Flower infection. Although the early workers recognized only seedling infection, Hoffman, to whom reference has already been made, as early as 1866 suggested the possibility of flower infection. His experimental inoculations were unsuccessful, however. Successful flower infection was obtained by Maddox in Tasmania in 1895 (See McAlpine, 1910) and by workers in Japan (Nakagawa, 1898; Hori, 1907), but it was really with the flower infection experiments of Brefeld (1905) that this type of smut infection became fully recognized.

Brefeld and Falck (1905) inoculated wheat and barley with their respective loose smuts by inserting a small brush laden with spores into the separate blossoms, or by blowing the spores inside a cylinder into the open flowers of the heads or panicles. As high as 96 per cent infection for wheat, and 99 per cent infection for barley was obtained by the individual blossom inoculation. All seedling inoculations with dry spores, soaked spores and infected compost were negative. When applied to the loose smut of oats, however, the above described methods of flower inoculation gave un-

satisfactory results, only a few isolated smutted plants being obtained. Falck (1908) continued Brefeld's experiments and finally succeeded in getting 50–80 per cent smutted plants by means of blossom inoculation with loose smut of oats.

Flower infection for the loose smut of barley (now *Ustilago nuda* (Jens.) K. & S.), and for the loose smut of wheat (now *U. Tritici* (Pers.) Rostr.) was further established by the work of Hecke (1904, 1905), Falck (1908), Lang (1910, 1917), and Broili and Schikorra (1913). Hecke demonstrated the mycelium of the loose smut of barley in the scutellum and plumule of the germinating seedling, while Broili and Schikorra showed it in the embryo of the resting seed previously inoculated at flowering time. Falck figured the mycelium of the loose smut of wheat in the embryo of the seed. Lang (1910) showed in the loose smut of wheat the course of the mycelium down from the stigma along the path of the pollen tube, then through the inner integument and nucellar tissue into the interior of the embryo. Further evidence (without any accompanying figures) for this method of penetration to the embryo was presented in a later paper (1917) for the loose smut of barley, and in addition evidence for penetration through the outer wall of the ovary.

Falck (1908) in the previously mentioned paper on loose smut of oats, examined the oat flowers after blossom inoculation and found that some of the spores had germinated on the stigma of the oat blossoms. He also found mycelium penetrating the outer layers of the flower infected seed, although there were also numerous ungerminated spores on the surface of the grains as well as on the inside of the glumes, and tests showed these spores wholly capable of germination. If the flower infected seed was disinfected before sowing, practically no smut resulted, the effectiveness of disinfection being explained by the fact that the spores remain on the outside of the ovary, and do not penetrate to the embryo as do *Ustilago nuda* and *U. Tritici*. They therefore are accessible to the disinfectant, whereas the mycelium of the two latter smuts is beyond its reach. Falck, therefore, considered the loose smut of oats as representing flower infection in its first phase, i.e. deposition of the spores on or between the floral parts. This type of flower infection is also suggested by Liro (1924).

Recently in Germany, the study of *Ustilago Avenae* (Jens.) K. & S. has again been taken up by Zade and his students, who lay special emphasis on the germination of the spores on the stigma of the oat blossoms. Falck, who was the first to report this in 1908, evidently did not consider it of primary importance in infection. However, these later workers (Zade, 1924; Neumeyer, 1924[1]; Arland, 1924; Diehl, 1925; Roesch, 1926, Tamme, 1927) believe that these germinating spores are the real cause of infection, forming

a resting mycelium and gemmae in the remains of the anthers and especially in the parenchyma of the glumes and infecting the young oat seedling in the following spring. Inasmuch as the spores get into the flower at blossoming time, they consider this a type of flower infection, but not in the sense understood by Brefeld, since in no case have they verified a penetration into the developing embryo. Their reason for believing this resting mycelium, rather than the ungerminated spores, is the infecting agent is based on their inability to secure high infection percentages by inoculating seed with dry spores. However, their attempts at flower inoculation have likewise proved unsuccessful. Neumeyer, and also Arland (1924) used a brush, Roesch (1926) a 'pulverizator,' Diehl (1925) suspended smutted panicles inside paper bags over the open flowers, and Tamme (1927) used a syringe without a canula, but none of these methods gave smutted plants in convincing numbers. In each case, in order to secure infection, intricate and unique methods of seed inoculation have been devised with the object of introducing resting mycelium between the glumes of hulled oats, or on the epidermis of naked kernels. Even then the percentages of infection have been in general very low.

Arland (1924) tried three methods: (1) by placing spores under the outer glumes of ripened kernels of hulled oats, (2) by dusting dehulled or naked oats with spores, (3) by washing fresh conidia in liquid in a Petri dish under the outer glumes. By the first method in ten varieties of *Avena sativa* he got no higher than 29 per cent infection, by the second method, 24.2 per cent infection; by the third method 5.1 per cent infection. The second method applied to *A. nuda* var. *chinensis* gave 78.5 per cent, and the third method applied to this same variety of oats gave 19.5 per cent.

Diehl (1925) removed the kernels from the glumes of the mature grain, sterilized and inoculated these glumes with spores, and incubated them for the development of resting mycelium. Each kernel was then returned to its original glumes, and stored. He got 39 per cent smutted plants as his highest infection.

Roesch (1926) sterilized strips of glumes instead of whole glumes and then proceeded according to the technique of Diehl. These pieces of glumes and also inoculated anther fragments were then inserted under the edge of the outer glumes down to the embryo of the grain to be tested. His highest amount of infection was 26.4 per cent.

Tamme (1927) dipped naked oats in a nutrient solution containing spores, and then kept them in moist Petri dishes with just enough moisture

[1] NEUMEYER, G. Studien ueber die Lebensweise und Bekämpfung des Haferflugbrandes, *Ustilago Avenae* (Pers.) Jens. Dissertation. Leipzig, 1924. Neumeyer's work is known to me only through the citation and comments of Zade and his students.

so that the spores germinated, but not the kernels. He obtained 18.5 per cent smutted plants. Hulled oats were inoculated by sucking up into a hypodermic syringe two or three drops of a dilution of spores in nutrient solution and then injecting this between the glume and the kernel. They were then kept, as were the naked oats, so that a resting mycelium might develop, and were later stored. Tamme obtained as high as 60 per cent infection with these hulled oats. The low percentage of smut in the naked oats of known susceptiblility is rather surprising, if the resting mycelium is the true cause of infection. Moreover, it is possible that ungerminated spores might have been a factor in securing the 60 per cent infection of hulled oats.

This 'new' type of flower infection has been obtained also in the case of *Ustilago levis* in addition to *U. Avenae* by Gage (1927), working in the United States. He inoculated oat blossoms artificially, very much after the method of Zade, by tapping a small camel's hair brush previously dipped in spores against the finger. It is interesting to note that he obtained as high as 67 per cent smutted plants with *U. Avenae*, and 67.5 per cent with *U. levis*, whereas Neumeyer and Arland found this method of inoculation to be unsuccessful to a large extent. According to Gage, the mycelium in the pericarp is accountable for the seedling invasion, and mycelium penetrating the glumes is insignificant, whereas the German workers hold the opposite view. Although both smuts may infect the flowers at pollination, the possibility of the spores of *U. levis* entering open flowers in the glumed varieties is very much less than for *U. Avenae*, because of the later time of spore dissemination in *U. levis*. Gage considers that in general the infection process in nature takes place principally through the open blossoms for *U. Avenae* and for *U. levis*, by means of spores reaching the caryopses of naked oats during threshing time. These in the latter case germinate in storage and produce the mycelium in or on the pericarp. His field experiments show high percentages of smut for both glumed and naked oats with both smuts inoculated at flowering time (over 60 per cent), and less than half that amount of infection when the method of inoculation was dusting dehulled kernels with spores, either at harvest time and then storing, or after harvest time. Gage believes that the mycelium developed in the pericarp grows directly into the mesocotyl of the seedling, since in seven day old seedlings he found mycelium only in the mesocotyl. However, no detailed cytological study of the seedlings was made.

Other smuts whose method of infecting their host plants has recently been brought into question are *Ustilago nuda* (Tisdale et al., 1924) and *Ustilago striaeformis* (Davis, 1924). Tisdale inoculated dehulled barley seed with spores of *U. nuda*, the loose smut of barley, which, as has already been

mentioned, was definitely established as a flower infecting smut by the researches of Brefeld, Falck, Hecke, Lang, and Broili and Schikorra. He secured 52–100 per cent smutted plants if the hulls were removed from the seed, but seedlings so treated showed signs of severe injury, which, however, Tisdale believed to be due to the smut and not to the dehulling. In a later paper (Tisdale and Griffiths, 1927) the suggestion is made that some strains of *Ustilago nuda* may be flower infecting and others seedling infecting.

Ustilago striaeformis was described by Osner (1916) as a flower infecting smut, but Davis (1924) comes to the conclusion that seedling infection is the common method. He atomized floral parts of timothy in all stages of development with nutrient solutions containing germinated and ungerminated spores, but failed to obtain smutted plants. He then subjected his spores to an after-ripening period of 250 days, and obtained 31 per cent infection when he applied a water suspension of germinated and ungerminated after-ripened spores to the seedlings. Seedlings were most susceptible when the coleoptiles were 1–10 mm. long.

Method of penetration of the fungus into the host plant

Microscopic evidence on the manner in which the smut fungus gains entrance into the host plant was presented by Wolff (1873) and by Brefeld (1895). Wolff described the sporidium as coming to rest with its usually somewhat swollen end firmly fixed on the epidermis of the leaf sheath and penetrating the cuticle and epidermal cell wall by means of a fine process which grows through the affected cell in a rapidly thickening hypha. Brefeld, on examining thin pieces of the epidermis of the leaf sheaths of oat seedlings under the microscope, found in three day old seedlings characteristic large holes in the cuticle. He states that

> Below this hole, which often reaches a considerable size, and proceeding from it inwards, can be seen the entering germ tube. Increasing in vigor (Ueppigkeit) and thickness, it crosses the outer cell layers and then loses itself in the deeper lying tissues until it can no longer be seen in tangential sections. Only at the most recent points of penetration is it possible to distinguish with certainty the conidium putting out the penetrating germ tube. It lies sometimes over the hole, sometimes alongside it, and loses its sharpness with progressive loss of its contents, until at the earlier points of penetration it is no longer to be distinguished. In favorable material the places of penetration are close together and are so numerous that the cuticle appears full of holes. . . . The points of penetration are most clearly recognizable on the third day after inoculation; here the germ tubes leading to the penetration holes still contain dense contents. Later, the points of penetration are less clear, and the tubes are drawn out into fine threads without contents, which can only be recognized as fungus hyphae by their connection with the deeper lying normal portions of the hyphae. (Translation.)

Similar holes to those described by Brefeld have been figured by Tisdale

(1924) and by Roesch (1926). Tisdale (1917), however, studying penetration of flax and cabbage seedlings by *Fusarium*, describes the diameter of the opening made by a hypha as somewhat less than the regular diameter of the hypha.

Lutman (1910) described the conidium closely applied to the epidermis with a progressive thickening of the host cell wall until the latter is passed and the germ tube makes its way into the protoplast through a small opening. He says that 'this continued dissolving and thickening of the walls produces the funnel-like openings of the conidial germ-tubes observed by Brefeld in surface view.'

Penetration through a small opening in the wall by means of a fine hyphal process is also described by Dastur (1920) for *Ustilago Sacchari*, the smut on sugar cane, and by Noble (1924) for *Urocystis Tritici*, the flag smut of wheat. In fungi other than the smuts, the most important cytological studies of penetration have been made by workers in Blackman's laboratory,—Blackman and Welsford (1916) for *Botrytis cinerea;* Dey (1919) for *Colletotrichum Lindemuthianum;* Boyle (1921) for *Sclerotinea libertiana;* and Waterhouse (1921) for the sporidia of *Puccinia graminis*. In all these cases, penetration by a minute cone or narrow process is described. Brown (1915, 1916, 1917, 1922a, 1922b) and Brown and Harvey (1927) have studied this subject of penetration from the physiological standpoint, and have come to the conclusion that the only satisfactory theory of membrane penetration by fungi is that the stimulus to penetration is one of contact, and that the means of penetration is purely mechanical, since there is no evidence of a cuticle dissolving enzyme. This work has been done principally with the fungus *Botrytis cinerea*, from which an extract was obtained which rapidly attacked the plant tissue when injected into it, but which had no action when simply placed on the surface. In support of the mechanical theory of penetration, through pressure, the ability of *Botrytis* germ tubes to penetrate paraffin membranes, and membranes of formalized gelatin, was demonstrated. *Eucharis* leaves showed no penetration even after several days, but when the tissue beneath the epidermis was scraped away, strong penetration after 24 to 36 hours took place through these 'epidermal windows.' This is taken to indicate that the total resistance to penetration is partly due to the turgor of the cells supporting the epidermis and partly to the cuticle itself. Successful penetration is accomplished by the minute rigid cone formed by the infecting hypha, and pressure being greatest at the apex of this cone, rupture of the epidermis takes place at this point.

Hawkins and Harvey (1919) present evidence in favor of the mechanical theory of penetration. In a study of the parasitism of *Pythium deBary-*

anum on the potato tuber, they found a correlation between resistance of the tuber tissue of various varieties to mechanical puncture and resistance to infection by the fungus.

The method of puncturing the host cell wall by means of a small opening is also described in haustorial development in the rusts. Allen (1923) and Rice (1927) describe a minute peg penetrating the host cell wall in the early stages of haustorium development, and Allen believes that an ultramicroscopic pore is formed in the wall of the fungus and the host cell wall next to it, probably by enzyme action.

Enzyme action is also mentioned by Roesch (1926) as the means by which the hole is formed in the cuticle of the leaf sheath of the oat seedling attacked by *Ustilago Avenae*.

Cytological evidence as to the relation of host and parasite

An exhaustive survey of the literature on the relation of host and pathogen has been made by Rice (1927). Therefore, I shall refer only to papers dealing with phases of the subject in which I am particularly interested.

Guttenberg (1905) described the cells of the sheath leaves of young ears of corn, parasitized by *Ustilago Zeae*. In no case did he find the fungus puncturing the plasma membrane of the host, but simply invaginating it. Such invagination was observed by Rice (1927) in the case of the corn rust haustorium, and by numerous other workers on fungi enumerated by her. Intercellular hyphae, intracellular hyphae, and haustoria which branch off from both the intercellular and the intracellular hyphae are described by Guttenberg. The haustoria are short, many branched and crooked.

Other workers who have described haustoria in the smuts are Fischer von Waldheim (1869), Wolff (1873), Clinton (1900), Lutman (1910), Anderson (1921), and Whitehead (1921). Von Waldheim figured 'Haftorganen' for *Ustilago Carbo* on oats, and for *Sorosporium Saponariae*, but his figures are not convincing (pl. 8, figs. 3, 10). He mentioned them as occurring also in *Tilletia endophylla*, *T. deBaryana*, *Ustilago longissima*, *U. Maydis*, and *U. hypodytes*. Wolff described much branched processes entering the interior of the cell and acting like haustoria for *Urocystis occulta* on rye (pl. 7, fig. 17). Lutman figured a haustorium of *Entyloma Nymphaeae* penetrating the leaf cells of *Nymphaea reniformis* (fig. 66). He does not describe haustoria for any of the Ustilagos studied (*U. Zeae*, *U. Tritici*, *U. nuda*, and *U. levis*). Whitehead (1921) figured branched haustoria in *Urocystis Cepulae*. Clinton (1900) figured well-developed haustoria of broom corn smut (now *Sphacelotheca Sorghi*) in pith cells from various parts of the plant.

A sheathed appearance of intracellular hyphae has been frequently de-

scribed in the literature on the smuts. Fischer von Waldheim (1869) described the mycelial strands as surrounded by a cellulose sheath which stained blue after an application of iodine and sulphuric acid, and belonged to the cell wall of the host. Wolff (1873) also described this sheathed condition of intracellular hyphae, and Guttenberg (1905) claimed that the sheath is a transformation product of the plasma membrane of the host cell formed with the cooperation of the host nucleus. Close proximity of the nucleus to the point of penetration when the hypha is about to enter the cell and to the hyphal filament inside the cell is taken as evidence of the activity of the host nucleus in the formation of this sheath. The haustoria are described as generally not sheathed by Guttenberg. Sometimes the hyphae appear as stretched and narrowed by the growth of the host cell, but the sheaths enclosing them are of normal thickness. The hyphae may appear as if ruptured inside the sheaths, and at these points the sheath is thickened and distinctly stratified. The host nuclei often appeared deeply lobed.

It is claimed by Lutman (1910) that the host cell at the point of penetration builds its wall thicker and thicker, the hypha meanwhile pushing through until finally the wall is passed. He shows a thickening of the host cell wall under the appressorium of *Entyloma Nymphaeae* (fig. 66), and a sheath-like investiture of the invading hypha developed from this appressorium, at the point of penetration of the host cell wall (figs. 64 and 65). The actual passage through the wall is described as a dissolving away of the wall. A similar interpretation by Lutman of the funnel-like openings observed by Brefeld has already been mentioned.

Brefeld (1895), however, interpreted this phenomenon as a swelling of the germ tube walls which extended to the penetrated cell wall of the seedling. He observed it in seedlings older than three days (plumules 1–2 cm. long), and described the germ tubes as not having the appearance of healthy, normal-growing filaments. That Brefeld was opposed to the idea of a cellulose sheath formation around the hypha is brought out by the following excerpt from an address delivered by him before the Klub der Landwirthe at Berlin in 1888 (my translation):

> The preparations which showed these figures had an unmistakable resemblance to Wolff's cases of penetration which he interpreted as showing that a cellulose sheath is formed around the penetrating hyphae. Such a sheath I have never seen in normal cases of penetration, and I am inclined to hold it all the more probable that Wolff saw only cases of arrested penetration with swollen germ tubes and gave to them the interpretation of cellulose sheaths, because he confined his infections to the leaf sheath alone, into which, in somewhat older stages, the germ tubes which have penetrated, can advance no farther.

Fellows (1928) in a study of the infection of wheat by *Ophiobolus graminis* reports as heretofore undescribed, penetrating hyphae surrounded by greatly thickened walls, to which he gives the name 'lignitubers.' He is apparently unaware of the similarity of his figures to the swollen germ tubes of Brefeld. In the epidermal cells of the coleoptile, he finds these 'lignitubers' always surrounding the penetrating hyphae, and frequently also in the cells of the root. He claims that by the use of microchemical tests he finds lignin to be the chief constituent of these abnormal appearing hyphae, and apparently considers that the host cell wall lays down this substance to stop the invading hypha. The cellulose sheaths of the early writers thus appear again under a new name.

Dastur (1920) described the hair cells on the bud scales of sugar cane as reacting to entering germ tubes of the sugar cane smut by developing a plug or thickening on the inner wall.

Important cytological studies of host-parasite reactions have been made recently in the rusts. Allen (1923a, 1923b, 1926a, 1926b, 1927) studied the reaction of susceptible and immune varieties of wheat to different physiologic forms of stem rust (*Puccinia graminis Tritici*), and to a form of the orange leaf rust (*Puccinia triticina*). She found that immunity does not consist in inability of the fungus to enter the stomata, for even in highly resistant varieties a high percentage of the germ tubes may gain entrance (52 per cent in Khapli emmer). On the other hand, resistance manifested itself more in an inability of the haustorium to develop fully without severe injury to the host cell. Mesophyll cells often are killed outright, and the haustorium as a rule does not develop beyond the early stages. Further attempts on the part of the fungus to establish relations with its host may result in formation of other haustoria which may expand and function for a while before the host cell is killed.

Characteristic conditions are noted in resistant hosts. The haustoria are covered with sheaths which are described as thick, irregular coverings greater in bulk than the haustoria themselves and enveloping both the body and the neck.

In form 9, on Khapli emmer (Allen 1926a) another type of reaction is the development of a heavy coating around the haustorium mother cells. This reaction was also noted in the susceptible variety Baart. A third type of reaction, observed in the resistant variety Malakoff (Allen 1927), is the production of wart-like prominences on the walls of living cells in the vicinity of the infection, but not in those cells closest to the fungus. Swollen host cell walls, differing from the above in that the entire wall is affected and not separate portions, are described in Mindum, resistant to form 3 of *Puccinia graminis Tritici* (Allen 1923b). The middle lamella is described as almost

liquified. Thickened host cell walls are also described in the resistant Kanred, where they occur between dead cells and living cells, walling off the pathological cells.

Rice (1927) made a study of the corn rust, *Puccinia Sorghi* Schw., on a susceptible variety of corn. She likewise noted thickened walls of infected host cells, especially at the place of penetration of the haustorium, thickened host cell walls near the limits of an infection area (in the region between the sori), and sheaths or heavy thickenings around haustoria. Allen seems to describe two phenomena, thickening to form heavy contact walls adjoining pathological cells, and swelling or decomposition of the lamellae due to a toxic enzyme produced by the fungus. Rice is unable to determine definitely whether her thickened host cell walls are due to secondary thickening or gelatinization, but she describes them as differing greatly from those described by Allen. As to haustorial thickenings, Rice did not observe any in the young flecks and in the 'green islands,' but she noted heavy sheaths in yellowed areas adjacent to these 'green islands,' and also in yellow areas around the sori generally. Thickening of the haustorial wall may therefore be a reaction set up by the host when there is a drain on it, as evidenced externally by yellowed areas.

Both Allen and Rice noted contacts between the haustoria and the host nuclei. Allen, however, interprets the nucleus as moving to the haustorium, whereas Rice suggests a positive tropism of the haustorium in relation to the nucleus, the significance of the contact involving absorption processes by the haustorium.

Ruttle and Fraser (1927), who made a study of *Puccinia coronata* CDA on a susceptible and on a resistant variety of oats, also noted thickened walls of haustorium mother cells and thickened necks of haustoria in the susceptible Banner, and thickened host cell walls in the resistant Cowra 35. The occurrence of the former in Banner, indicates a slight resistance in this otherwise susceptible host. The thickenings of the host cell walls in Cowra are similar to those described by Allen.

Physiologic forms and heterothallism in connection with the host-parasite relation

In 1921, Zillig presented evidence for the existence of some eight specialized forms of the anther smut, *Ustilago violacea*, on different species of *Dianthus*, *Melandryum*, and *Saponaria*, which he distinguished from each other mainly by their pathogenicity for certain members of the Caryophyllaceae, and their inability to infect others. The number of plants used in his experiments on cross infection, however, was often not more than five or ten, and in most cases less than fifty was the maximum, so that his

results, although suggestive, do not entirely justify the conclusion that the different forms of *Ustilago violacea* on different members of the Caryophyllaceae are limited strictly to those hosts. It is, moreover, of special interest, that in this flower smut, flower and shoot inoculation proved unsatisfactory, and seedling inoculation was practiced, as the usual method of procedure. This method, although giving low percentages of infection in a great many cases, did infect 62 out of 69 plants of *Dianthus Carthusianorum* (89.88 per cent) when smut from this host was used as inoculum. Whether different physiologic forms may gain entrance to the host in different ways (i.e. some flower infecting, others seedling infecting) is a question raised by Tisdale and Griffiths (1927) in connection with their seedling infection of barley with *U. nuda*, and might also be brought up in connection with these results of Zillig.

Testing pathogenicity of different smut strains on different host varieties in the cereal grains by Faris (1924a, 1924b) and Reed (1924a, 1927, 1928) gave additional and more conclusive proof of the existence of physiologic forms in the smuts. Faris identified by testing infection on various varieties of barley, five physiologic forms of *Ustilago Hordei* which he obtained from various localities, and Reed (1924a, 1927) identified four forms of *U. Avenae*, one from Missouri, another from Wales, and the other two collected from various places in the United States, and differentiated by their capacity to infect hitherto resistant varieties of oats. He also identified by infection experiments two physiologic forms of *U. levis*. Physiological specialization in bunt, suggested by Reed (1924b) and indicated by Faris (1924c), was confirmed by the identification (Reed 1928) of four physiologic forms of *Tilletia laevis*, and six of *T. Tritici*. Additional tests (Reed 1930) bring the total number of specialized races of *U. Avenae* to eleven, and of *U. levis* to five. Physiologic specialization in *Tilletia* was also suggested by Rodenhiser and Stakman (1927) and by Gaines (1928) and in *Ustilago nuda* by Tisdale and Griffiths (1927). Three physiologic forms of *Sphaceolotheca Sorghi* indicated by Tisdale, Melchers, and Clemmer (1927) have been shown to differ in culture by Ficke and Johnston (1930). In all the experiments of Reed and Faris, inoculations were made with dry spores, and infection was obtained apparently from conidia.

In addition to differences in pathogenicity, the existence of physiologic races in smuts has also been claimed on the basis of differences in cultural characters, such as rate of growth on culture media, color, relative amount of aerial mycelium, nature of mycelial growth, topography, zonation, surface, character of the margin, and abundance of conidia. Christensen and Stakman (1926) report the differentiation of fifteen forms of *Ustilago Zeae* by this method, and Rodenhiser (1926, 1928) reports twelve forms of *U.*

nuda, fourteen forms of *U. Tritici*, seven forms of *U. Hordei*, five forms of *U. levis*, and 18 forms of *U. Avenae*. Eight of the physiologic forms of *U. Zeae* so distinguished differed also in their pathogenicity for ten selfed lines of corn, and differences in pathogenicity were noted when two of the seven forms of *U. Hordei* were tested by Rodenhiser on six varieties of barley.

That new physiologic forms may arise as wedge-shaped sectors appearing spontaneously in colonies developing on plate cultures of conidia, is maintained by Christensen and Stakman (1926), and they consider these culturally different and also pathogenically different forms as mutants. Mutant sectors, showing difference in color and rate of growth from the parent cultures, were also obtained by Rodenhiser (1928) in cultures of *U. Hordei* and *U. Avenae*. He, however, suggests that these sectors may indicate not necessarily mutation from a single physiologic form, but the presence of more than one physiologic form in the culture, since the original isolations were not made from a single chlamydospore but from a mass culture of chlamydospores of a single collection. He also suggests that they may be sexual strains, the conidia of one sex giving rise in culture to a different type of growth than those of the other sex. The question whether these changed forms are true mutants is raised by Goldschmidt (1928), because their constancy has been tested only in the vegetative condition. In order to be a true mutant, the changed form must be maintained in the offspring following a sexual union. Its cause must be a permanent inheritable change originating in the germ plasm. Purely vegetative propagation with its equational division at each mitosis might carry on by cell inclusion a change which appeared permanent throughout the successive cell generations, but which was not constant in the sense of a permanently altered germ plasm. From this standpoint, it would hardly be possible to apply the term mutant to any of the apparently permanent changes either physiological or morphological arising in bacterial cultures, or to the bud variants or sports on plants which do not set seed, although in both cases the term mutant is frequently applied in the literature of today. These conidial cultures of the smuts are comparable to bacterial cultures in that they reproduce exclusively in an asexual manner.

Intimately associated with this study of physiologic forms in culture is the evidence that some physiologic forms may be sexual forms obtained by a segregation of conidia. Stakman and Christensen (1927) found that four culturally different physiologic forms of *Ustilago Zeae* with which they had not been able to cause infection in field experiments hitherto, were able to infect Golden Bantam corn, provided each was used in combination with a culture designated as M2, which also was unable to cause infection when used alone. Additional cultures from three localities, New Hampshire,

Mississippi, and Canada, unable to infect when used alone, or in combination with each other, were able to infect when used with M2. This evidence is taken to indicate that physiologic form M2 was of one sex, while all the other forms were of the opposite sex. It is presented as evidence of heterothallism in the smuts, a condition suggested by Kniep (1919), although he recognized difficulties in fitting the smuts into the hetero-homothallic scheme. In his recent book (1928) he uses the terms monoecious and dioecious in place of homothallic and heterothallic, in discussing the smuts. He states that a precise answer cannot be given as to whether they are monoecious or dioecious. Copulating sporidia indicate the fungus is dioecious, whereas copulating cells of one promycelium indicate monoecism. 'Monoecious' and also 'dioecious' as used by Kniep refer only to the 'phenotypic picture'; the genotypic constitution of the nuclei as regards sex is indicated by the term 'heterokaryotic' or 'homokaryotic.' In monoecism, then, Kniep recognizes two possibilities: (1) a heterokaryotic condition in which there are two kinds of nuclei with genotypically different sex realisators, which separate inside the individual, giving rise to sperms and eggs; (2) a homokaryotic condition in which all the nuclei are genotypically identical. The *Anlage* of both sexes are in each nucleus, and unknown factors in individual development decide on the development of these *Anlage*. The nuclear condition in the promycelium of the Ustilagos illustrates the first condition, i.e. the heterokaryotic; it is 'haplomonoecious' and a 'miktohaplont.' The term 'homothallic,' at least in the Mucors, is confined by Kniep to the homokaryotic condition above described. It seems as though this new terminology were making the situation unnecessarily complicated, and was not really offering any solution to the question at hand. If Kniep believes, as he undoubtedly does, that a reduction division takes place when the chlamydospore germinates, and that sexually differentiated cells result at this reduction, then it is difficult to see why he hesitates to call the smuts heterothallic (or dioecious), since it is generally accepted that this sex differentiation at reduction division gives rise to the heterothallic condition, and the delaying of sex differentiation until some later time in the life of the haplont that gives rise to the homothallic condition. The mature promycelium of the smuts might then be compared to a spore tetrad of *Sphaerocarpos*, if it is accepted that sex differentiation of nuclei has taken place in the two nuclear divisions in the young promycelium. Although the cells remain in continuity, the organisms produced by each cell are separate gametophytes.

Recently evidence has been presented by Eddins (1929) for homothallism in the smuts. He obtained smut galls of *U. Zeae* when he inoculated corn plants with a monosporidial culture of this smut. The significance of this will be taken up later in my discussion.

The necessity for combining two unisexual cultures in order to secure infection, besides suggesting heterothallism, also suggests to Stakman a second possibility in addition to mutation in culture as to the origin of new physiologic forms, i.e. by hybridization, involving the pairing of conidia from different species of smut. Liro (1924) suggested this when he described two forms of *U. Avenae*, 'Weichbrand' and 'Hartbrand,' the former producing very loose spore masses, the latter forming compact spore masses with glumes of the host plant unaffected, as in *U. levis*. Inoculations with 'Hartbrand' gave plants smutted with 'Weichbrand.' Hybrids between *U. Avenae* and *U. levis* are suggested as a cause of this condition.

Dickinson (1927a, 1927b) also suggests the possibility of hybrid smuts. He tried infection experiments with monosporidial cultures of *U. levis* and *U. Hordei*, and found that cultures of sporidia of the same 'gender' would give rise to mycelia unable to penetrate the epidermis of the coleoptile, whereas cultures of sporidia of two 'genders', applied simultaneously to the coleoptile, whether the two 'genders' were from one species (*U. levis*) or from two different species (*U. levis* and *U. Hordei*), gave rise to mycelium capable of penetrating into the underlying tissues of the seedling in 91 per cent of the seedlings examined (186 out of 204). The possibility of a hybrid between *U. levis* and *U. Hordei* is thus suggested. It is open to question whether penetrating mycelium in seedlings as young as those examined by Dickinson would give conclusive evidence of successful infection.

The actual production of hybrid smuts obtained by inoculating species of the Caryophyllaceae with sexually different sporidia from different physiological forms of the anther smut is claimed by Goldschmidt (1928). The promycelium of the hybrid spores, when compared with that of both parents, is larger than either. A genetic analysis of at least one of these hybrids by 'back crossing' the isolated sporidial strains with each parent is claimed by Goldschmidt as showing physiologic specialization to be due to a Mendelian gene.

Recently Hanna (1929) has reported that there may be not only two but also four sexual types of sporidia in *Ustilago Zeae* and *Sorosporium Reilianum*, thus extending Kniep's (1920) idea of multisexuality in the Hymenomycetes to the Ustilaginales. The presence of more than two sexual types among smut conidia was first reported by Bauch (1923) in his three sex forms in *Ustilago longissima*, but the actual harmonizing of these results with the idea of multipolar sexuality in the Hymenomycetes has been reported by this author only within the last year (1930). *Ustilago longissima*, according to Bauch, shows besides the well-known bipolar sexuality, a tetrapolar, which means that there are four different groups of haplonts, due to the presence of two factors for sex. Only haplonts in which

both factors are different show a complete sexual reaction (copulation of sporidia with formation of long germ tubes—'Suchfadencopulation'), but there occurs also a different type of copulation called 'Wirrfadencopulation' between sporidia alike in one sex factor. It is because these two types of copulation were not recognized in the earlier work that the existence of tetrapolar sexuality was not suspected, and three 'sexes' were reported, when now as many as twenty-four sexes (i.e. sexually different haplonts) are recognized, due to the presence of multiple allelomorphs of both sex factors. The 24 sexes above referred to, fall into three sex groups, which correspond to the three sexes first reported, if the presence of 'Wirrfadencopulation' is ignored.

Hanna holds views similar to Bauch on the sexual character of the sporidia in the smuts, but it is open to question whether sex is the underlying condition here. Brunswik (1924) takes the stand that the Mendelian factors governing copulation are not sex factors as held by Kniep, which by their presence occasion copulation, but rather sterility factors which, arising originally in homothallic forms, prevent copulation. This puts the discussion of fruit body formation in Hymenomycetes and spore production in smuts in the same class with self sterility or self incompatibility in the flowering plants, where there is no question of the presence of the two sexes, but where there is often a failure to fertilize and produce seed. Stout (1920, 1923) has studied such incompatibilities in various flowering plants (Chicory, *Brassica pekinensis*, day lilies, wild apples, etc.), and he holds that such incompatibilities arise 'primarily in the ontogenetic processes of physiological differentiation of sex organs, and are not determined by either individual stuffs or line stuffs of definite hereditary value' (1920). Similarity in parents according to Stout favors fertility (as far as numbers are concerned). Although Kniep has changed the term sex factor to copulation factor (1928), he firmly maintains that absence of copulation is due to the presence of like copulation factors and not to sterility factors such as those of the higher plants.

Hanna's investigations (1929) are a continuation of the work of Stakman and Christensen (1927) already mentioned, and are based on infection experiments with sporidial cultures originating from individually isolated sporidia. Since the sporidia of *U. Zeae* are not known to conjugate, gall production on the host plant is made the basis for deciding whether these sporidia are sexually alike or sexually different. Microscopic study of the epidermis of an inoculated leaf showed in one case two sporidia lying close together sending out germ tubes which penetrated the epidermal cell. These slender germ tubes came together and formed a stout diploid hypha in which several pairs of nuclei could be seen. Thus in *U. Zeae* it is sug-

gested that the binucleate condition originates by the fusion of germ tubes which have penetrated the host. Hanna's results are in direct contrast with those of Zillig, Goldschmidt, and Dickinson, all of whom report for the smuts studied by them that the haploid mycelium (from an unconjugated sporidium) cannot penetrate the host.

Along with these infection experiments on smuts, should also be mentioned perhaps, Craigie's claims (1927a, 1927b, 1928) that the sporidia of *Puccinia graminis* and *P. Helianthi*, and also the pycnospores (spermatia) are of two sexes, and that they must be combined in order to get aecia on the host.

Dodge (1924, 1926) reports the frequent absence of spermogonia coupled with the development of uninucleate aecidiospores which germinate into two-celled promycelia, for a strain of the short-cycled form of the orange leaf rust, *Caeoma nitens*. He suggests the possibility that this is a plus strain and that the orange leaf rust is heterothallic. There is no cell fusion in the formation of the uninucleate aecidiospores, and Dodge suggests that they are produced by parthenogenesis. The absence of the male strain is indicated in the lack of spermatia. The much rarer cases in which spermatia are present with uninucleate aecidiospores indicate an androgenetic strain. In both cases, aecia are produced. The occurrence of spermatia is then an expression of maleness, and the male character of the original infecting sporidium. Evidently the heterothallism suggested by Dodge's results is quite different from that found by Craigie. Craigie's plus and minus strains both produce spermatia, and neither produce aecidia alone. Dodge's strains may both produce uninucleate aecidiospores, but only one, the male, produces spermatia. Craigie, however, believes that even the aecidia which may sometimes appear later on without spermatial mixing do not produce uninucleate spores, as he at first reported, and as they perhaps should, if they are parthenogenetic, but produce binucleate aecidiospores. The situation in the smuts is quite different Here there is nothing which can give any clue to the identity of a male or female strain, as do the spermatia in the rusts. The idea of sex and heterothallism in the smuts is based wholly on the fact that the conidia fuse, and the possibility of parthenogenetic and androgenetic development is in general disregarded.

RESULTS OF ORIGINAL INVESTIGATIONS

Material and methods

Kernels of *Avena sativa* var. Victor (S.N. 126), a susceptible variety, were inoculated with spores of *Ustilago Avenae* (Pers.) Jens. from the previous summer, by the dry spore dusting method. The smut used was a

strain originally obtained from Missouri by Dr. George M. Reed of the Brooklyn Botanic Garden. The method used was to place the seed (after the hulls had been removed) in a small envelope with a sufficient quantity of spores to dust it thoroughly. The seed was then planted in sand, one inch below the surface, germinated at room temperature, and after the seedlings were above the surface, they were transplanted to soil in greenhouse benches. The moisture content of the sand was 15 per cent of its total water holding capacity. This moisture percentage and temperature for germination were used because data previously obtained showed them to be very favorable for the production of smut (Reed and Faris, 1924). Lots of sixty seeds were inoculated; about 30 seedlings were grown to maturity on the greenhouse benches, and the same number was used for cytological study at different stages of their development. This procedure was repeated so that four plantings of Victor were made at different dates —November 28, December 11, 1924, January 5 and January 15, 1925, and also a planting of Black Mesdag (S.N. 70), an immune variety, which has so far never shown any smut. Slight variations from the above general procedure are indicated in table 1.

TABLE 1

Percentage of smut in plants raised to maturity

VARIETY OF OATS	DATE OF PLANTING	MOISTURE PER CENT	TEMPERATURE	PRESENCE OR ABSENCE OF HULLS	NO. OF SMUTTED PLANTS	NO. OF SMUT FREE PLANTS	PER CENT SMUT
Victor 126	11/28/24	15	Room	Hulls removed	29	0	100
Victor 126	12/11/24	15	20° tank	Hulls removed	22	0	100
Victor 126	1/ 5/25	15	Room	Hulls removed	29	0	100
Victor 126	1/15/25	15	Room	Hulls not removed	30	0	100
Black Mesdag 70	1/26/25	15	Room	Hulls removed	0	30	0

For microscopic examination of living material, a small piece of the inner epidermis of the coleoptile was stripped off, and a small strip of the epidermis of the mesocotyl. This was mounted in water or lacto-phenol and examined under an oil immersion lens. Staining this fresh material was not necessary, as the fungus hyphae, when present, could be readily detected in unstained preparations.

From the time these seedlings emerged above the sand (usually about the 4th or 5th day after planting) until they were about 15 days old, a few were examined daily by the method noted, and totals of these results are recorded in table 2. For example, from January 5 (1925) planting, one seedling 4 days old, seven seedlings 5 days old, five 8 days old, ten 9 days old, six 10 days old, and two 12 days old were examined. The age of the seedling is reckoned from the day of planting.

After examination in the fresh condition, portions of the seedlings usually one half to three quarters of an inch long, including as much of the plumule and mesocotyl above and below the coleoptile node as possible, were fixed in a mixture of Flemming's weak and medium fixatives, embedded in paraffin, sectioned, and stained with Flemming's triple stain. In a few cases Heidenhain's iron-alum haematoxylin stain was used. Portions of the seedling including the root node and the mesocotyl, and others taken from

TABLE 2

Location of mycelium in seedlings studied

VARIETY OF OATS	DATE OF PLANTING	NO. OF PLANTS EXAMINED	NO. OF PLANTS FREE FROM MYCELIUM	LOCATION OF MYCELIUM	
				COLEOPTILE ONLY	COLEOPTILE AND MESOCOTYL
Victor 126	11/28/24	30	3	19	8
Victor 126	12/11/24	23	0	3	20
Victor 126	1/ 5/25	31	0	3	28
Victor 126	1/15/25	29	1	5	17
Black Mesdag 70	1/26/25	30	0	29	1

the tips of the coleoptile downward were also fixed. Chromacetic acid, and Bouin's fixative were also used, but the Flemming's fixative gave the best results. Sections 10 microns and 15 microns thick were found best for the study of the mycelium, except the growing point, where sections 5 microns thick were found to be better.

In order to get material less than five days after inoculation for cytological study, seed with hulls removed was plentifully dusted with smut spores and germinated on moist filter paper in Petri dishes at room temperature. Seed was also planted in moist sand, about one inch below the surface, and then dug up, washed free from sand particles, and the scutellum dissected out from the endosperm so that the entire seedling could be fixed whole, or in two parts, scutellum, root node, and lower portion of the mesocotyl constituting one part, and the upper portion of the mesocotyl, coleoptile node, and plumule to its tip constituting the other part.

Anatomy of the young seedling

By mesocotyl, I refer, as do Sargent and Arber (1915), to that portion of the axis of the young oat seedling between the insertion of the scutellum and the insertion of the coleoptile.[2] Zade (1918) refers to it as the hypo-

[2] The use of the term mesocotyl has been criticized recently by Avery (Bot. Gaz. **89**: 1–39. 1930) who considers this region an internode. In my paper, the term is retained regardless of the morphological interpretation of the region, for much the same reasons that probably have led others to retain it, that is, as a convenient method of

coleoptile. In the ungerminated seed, it is undeveloped, but when the seed germinates, it varies in length with the depth of planting; the deeper the seed lies beneath the surface of the soil, the longer the mesocotyl becomes. It may also be described as the part of the axis between the primary root node and the coleoptile node.

The mesocotyl contains a central mesocotylar stele which is prolonged into the primary root, and the scutellum bundle which turns into the mesocotyl at the point of insertion of the scutellum and runs parallel with the central stele of the mesocotyl, but distinct from it, up to the coleoptile node.

The coleoptile is the sheath that encloses the plumule. In the oats I have studied, it contains two vascular bundles on opposite sides of the seedling. The tissues in cross section show a layer of outer epidermal cells quite heavily cutinized, especially near the tip, six to eight layers of parenchyma cells, and a layer of inner epidermal cells also cutinized. Stomata are present in the outer epidermis. Plastids develop in the outermost layers of the parenchyma cells.

Percentage of smutted plants and microscopic examination of seedlings

The four plantings of Victor (S.N. 126) totalling 110 plants, gave 100 per cent smutted plants (see table 1). The 30 plants of Black Mesdag were entirely smut free. This variety of oats had in previous experiments consistently proved itself immune (Reed, 1925a). The hulls were removed from the seed in the case of three of the plantings of Victor and for the planting of Black Mesdag. In the single planting of Victor in which the hulls were left on the kernels, the amount of smut was not reduced. However, it has been found in other experiments that removal of the hulls increases the percentage of smut in susceptible varieties, but does not affect it in such resistant varieties as Black Mesdag.

The object of the plantings was to make possible a comparison of the number of diseased plants at maturity with the number of seedlings showing the presence of mycelium. Microscopic examination of seedlings (living material) ranging in age from 4 days to 15 days showed the mycelium had penetrated into the host in the case of 109 of the 113 seedlings of Victor which were examined (see table 2). Probably the failure to find the mycelium in the four cases was due to oversight. This preliminary examination of seedlings also gave information as to the distribution of mycelium in the parts of the seedling (see table 2). In all seedlings except six, mycelium was found in the cells of the inner epidermis of the coleoptile;

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designating a portion of the axis of the seedling of great importance in the germination of corn and oats, without necessarily subscribing to the theories which the originator of the term (Celakovský) may have had as to the morphology of the embryo.

these six seedlings which belonged to the series of January 15, 1925, with hulls, showed mycelium only in the epidermal cells of the mesocotyl. In many seedlings (73 in number) mycelium was also found in the epidermal cells of the mesocotyl in addition to being found in the cells of the inner epidermis of the coleoptile. The hyphae were however so wide-spread that evidence as to the place of original penetration of the fungus in any of these seedlings was difficult to obtain, and it immediately became apparent that younger stages would have to be studied to determine this point.

The small number of cases in the planting of November 28, 1924, in which mycelium was found in both coleoptile and mesocotyl was due rather to incomplete examination of the mesocotyl, since this experiment was the first of the series. I examined only a single piece of epidermis, whereas in the later series, negative findings were not taken as conclusive until after four or five pieces of epidermis had been studied.

It is interesting to note that all the seedlings of Black Mesdag gave unequivocal evidence that mycelium was present in the coleoptile. The resistance of this variety, therefore, is not due to failure of the fungus to penetrate the coleoptile. Kühn (1874) as noted, regarded the penetration of the coleoptile as of little significance. Recently Reed (1925b, 1928b) has shown that the resistance of Black Mesdag crossed with Victor is inherited in Mendelian ratio.[3] Garber, Giddings, and Hoover (1928) confirm this, but they also believe there is at least one supplementary factor brought in by the Black Mesdag parent in a cross of Gopher by Black Mesdag which causes transgressive segregation for susceptibility, since F_3 and F_4 families were obtained in which a distinctly greater percentage of smutted plants was found than in any of the plots of the susceptible parent.

I have studied especially the seedlings of the variety Victor and am presenting the results of my study of fixed material in somewhat diagrammatic figures. The spores used were a strain of *Ustilago Avenae* originally obtained from Missouri by Dr. George M. Reed.

Place of entrance of the fungus into the seedling and distribution of the mycelium

Text figures 1–4 are semi-diagrams of seedlings germinated in Petri dishes at room temperature, figures 5–9 are semi-diagrams of seedlings grown in sand, planted about one inch below the surface. In all cases, the

[3] Both these parents react in the same way to both loose and covered smut, that is Black Mesdag is resistant to both, whereas Victor is susceptible. More recently Dr. Reed reports (Brooklyn Botanic Garden Record, March, 1931) testing the inheritance of resistance in hybrids from parents which do not react in the same way to the two smuts. Early Gothland is susceptible to loose smut, resistant to covered, while Mon-

complete series of longitudinal sections was carefully studied in order to locate the mycelium. Figures 10–15 are cross sections through a five days old seedling, figures 16–19, of an eight days old seedling.

In view of the claims for flower infection by *U. Avenae* made by Gage and also by Zade and his students, it seems wise to include, in this study of penetration of the host plant, the results of the study of seedlings in younger stages than that in which penetration was commonly found to take place. Such seedlings (one and two days old) showed with one exception no penetration through the coleoptile or any other organ, as I will presently demonstrate. They were also examined for any evidence of mycelium which might indicate the presence of the pathogen within the embryo before the seed germinated, but in no case was any mycelium found.

Figure 1 shows a median section through a one day old seedling. Many spores were noted, closely adhering to the surface of all parts of the seedling—the coleoptile, coleorhiza, young root epidermis, and the mesocotyl, but only one case of penetration in any one day old seedling studied was observed at the point marked × on the coleoptile. The germ tube, which was very slender and faintly stained, had not penetrated beyond the epidermal cell. Directly below the point of penetration lay a spore with its promycelial tube closely applied to the cuticle, but not penetrating. Many of the spores showed promycelia.

Two day old seedlings were studied but no perforations and no mycelium could be found in them. Many of the spores adhering to the epidermis of the coleoptile had germinated, producing promycelia and numerous conidia. This would seem to indicate that a certain stage must be reached by the host before penetration can take place.

Figures 2 and 3 show two diagrams from a three day old seedling, figure 2 representing a tangential section, showing one of the bundles of the coleoptile, and figure 3 showing a median section through the seedling. Places of initial penetration along the epidermis of the coleoptile where the germ tubes were just entering the epidermal layer are marked ×, and were very numerous. A number of these were very favorable for the cytological study of penetration. Some of the invading tubes, especially those in figure 3, showed signs of being arrested in their growth. They were funnel-shaped, thicker walled than ordinary hyphae, and often the cytoplasmic contents were invisible or reduced to a bluish stained strand. A case of what ap-

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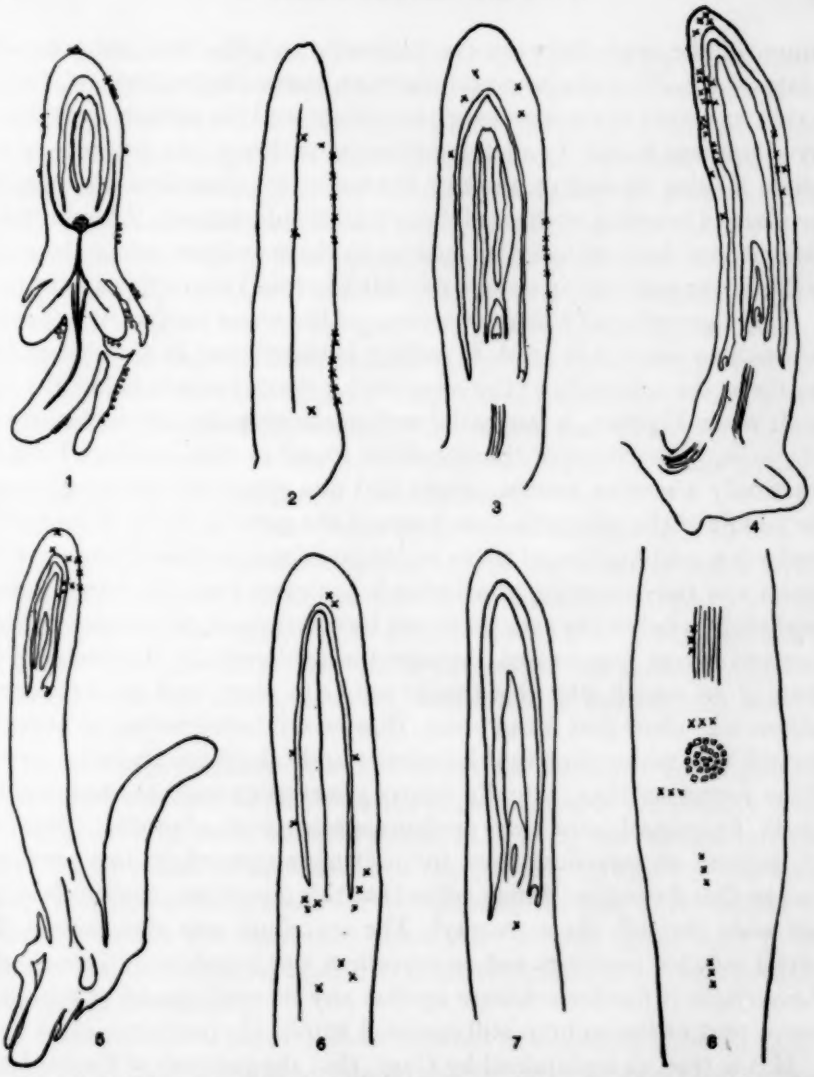
arch is resistant to loose smut, susceptible to covered. Here results indicate that the factors determining resistance to the two smuts in the hybrids are independent of each other. This complicates the situation relative to a Mendelian explanation for inheritance of resistance.

peared to be penetration of a promycelium direct from a spore (plate 27, fig. 1) was noted down near the coleoptile node as shown in text figure 2. Other ×'s in the diagram represent the location of bits of mycelium which had already penetrated to the deeper tissues of the seedling (parenchyma and vascular tissue). Another three day old seedling of this series showed no initial penetration, that is no penetration into the epidermal cells, but showed mycelium already in the parenchyma up near the tip of the coleoptile. The ×'s in the upper part of the diagram of the coleoptile in figure 3 represent the location of this mycelium. As there was no indication of mycelium elsewhere in this seedling, the fungus must have gained entrance through the coleoptile.

Figure 4 is a diagram from a section of a seedling 4 days old. Mycelium as indicated (×) was plentiful in the upper portion of the coleoptile, extending to the inner epidermis, and in a few cases it was found in the first leaf. Very long hyphae were found growing downwards towards the growing point in the space between the coleoptile and the first leaf, some of these having reached a distance approximately midway from the tip of the coleoptile to the growing point as illustrated in the diagram. No penetration into the epidermal cells was observed in this seedling, but the distribution of the mycelium indicates that the tip and a place on the right side of the diagram, where the mycelium is isolated from that on the left side, were the points of original entrance. No penetration into the epidermal cells was found nor any mycelium at the primary root node or in the mesocotyl of this 4 day old seedling.

As indicated in figure 4, very little development of the mesocotyl was found in seedlings germinated in Petri dishes. The seedlings planted in soil all showed well developed mesocotyls. Only in the case of the one seedling, a section from which is diagrammed in figure 5, was it possible to fix the whole seedling in one piece. This seedling with others was grown from seed dusted with dry spores, each seed then being placed in a small gelatin capsule about half an inch long with open ends, and smut spores were packed closely around the kernel, filling the capsule. This procedure was followed in this special case with the hope that many cases of initial penetration might be obtained for cytological study. This, however, did not turn out to be the case.

In text figure 5 is shown the section of the seedling above referred to, which was slightly less than three days old. Penetration through a hole in the cuticle, with a swelling of the germ tube when inside the epidermal cell (plate 27, fig. 5) was found up near the tip of the coleoptile on the right hand side of the diagram, but mycelium already in the parenchyma had made its way to the inner epidermis of the coleoptile. The presence of my-



Figs. 1–8. Diagrams of sections through oat seedlings of various ages, inoculated with the loose smut of oats, *Ustilago Avenae*. ×'s show the location of fungus hyphae. Fig. 1. Longitudinal section through a seedling one day old, germinated on moist filter paper in a Petri dish, inoculated by the dry spore dusting method. Numerous spores are shown adhering to the seedling. × shows the location where a single germ tube has entered the epidermis of the coleoptile. Fig. 2. Longitudinal section through the coleoptile in the region of one of the coleoptile bundles, of a seedling three days old. Fig. 3. Median longitudinal section through the same seedling. Fig. 4. Longitudinal section through a seedling four days old. Fig. 5. Longitudinal section through a seedling two and a half days old, from a kernel inoculated and placed in a gelatin capsule, and planted in sand. Fig. 6. Longitudinal section through coleoptile and first leaf of a seedling four days old, inoculated and germinated in sand. Fig. 7. Median longitudinal section through the same seedling. Fig. 8. Tangential longitudinal section through a seedling thirteen days old, showing cross section of a nodal root at the coleoptile node.

celium in the space between the coleoptile and the first leaf was noted (plate 30, fig. 22), and also mycelium was observed in the first leaf. Careful search was made of the mesocotyl, scutellum, and the primary root, but no mycelium was found. In all these younger seedlings, the presence of mycelium making its way up through the mesocotyl from the root node, and the place of insertion of the scutellum trace would suggest, if found, a possible internal focus of infection such as in the scutellum before the germination of the seed, but in no case could it be proved that this had occurred.

Text figures 6 and 7 show two views of the upper portion of a four day old seedling planted in sand. Mycelium is distributed in the tissues from the tip of the coleoptile to the mesocotyl a short distance below the coleoptile node. Figure 6, a tangential section showing the first leaf inside the coleoptile, shows most of the mycelium found in this seedling. Figure 7, practically a median section, shows that one hypha has gotten almost to the middle of the coleoptile node beneath the growing point. Whether this hypha is a continuation of those whose position is indicated in figure 6 at the base of the coleoptile, or whether it has come from those in the mesocotyl slightly below the node could not be determined. Although I have not observed direct penetration through the epidermis of the mesocotyl, I know of no reason why this should not take place, and further investigations may show that it can occur. However, it is interesting to note that Brefeld, who maintained that infection could take place through any part of the young seedling, actually figures penetration only through the leaf sheath. Examinations of lower portions of mesocotyls of seedlings four days old, showed no mycelium from the root node upward, in this particular case, so that I consider it impossible that this mycelium came up from the root node through the mesocotyl. The scutellum was also examined in several infected seedlings and no mycelium was found in it. This absence of mycelium is further evidence against any internal spread of mycelium from a part of the embryo still enclosed within the pericarp.

If it is true, as maintained by Gage, that the pericarp of the seed harbors the resting mycelium which actually causes infection, it is surprising that this mycelium, placed so favorably in relation to the parts of the embryo, does not appear in the parts of the young seedling sooner than is reported by him. He did not find mycelium in seedlings younger than seven days old, and then only in the mesocotyls. From the data already presented in this paper, it appears that any ungerminated spores on the outside of the grain can germinate and penetrate through the coleoptile deep into the underlying tissues of the seedling before it is five days old. In fact one of the first difficulties encountered in this problem of finding initial penetration of the epidermis was due to the fact that seedlings from five days old

through to those 13 days old were studied first, and in them the mycelium was so widespread throughout the tissues of coleoptile and mesocotyl that it was impossible to gain any clue as to the original point of entrance. Such a five day old seedling cut in cross section showed mycelium equally distributed above, below, and in the tissues of the coleoptile node (text figs. 10–15). It was especially abundant in the coleoptile in the vicinity of the two vascular bundles, in the parenchyma cells of the node between the bundles from the leaves, and near the point of origin of the nodal or crown roots, and also in the cortical tissue of the mesocotyl. An 8 day old seedling, also cut in cross section, likewise showed mycelium about equally distributed in all sections of the mesocotyl and the coleoptile (text figs. 16–19). Longitudinal sections of the lower portion of an 8 day old seedling, including the primary root node and the lower portion of the mesocotyl, showed mycelium in the epiblast and in the mesocotyl.

Figures 8 and 9 are made from longitudinal sections of a 13 day old seedling. They show mycelium abundant near the vascular tissue and in the vessels, both in the coleoptile and in the mesocotyl. Much mycelium was also found in the region of the node, in the axils of the leaves, and at the base of the coleoptile. The bud in the axil of the coleoptile showed mycelium in its tip. None, however, was noted in the growing point of the main stem.

This seedling, therefore, although too old for a study of initial penetration, showed very well the distribution of the hyphae in the vicinity of the growing point. The paths followed appear to be along the vascular bundles, and although actual establishment in the growing tip had not yet taken place, nevertheless the presence of mycelium in the bud, in the axil of the coleoptile, and in the tissue just below the tip (base of the next to the youngest leaf primordium) should be sufficient to insure an early invasion of the actual growing tip.

Lutman found the growing point infected in a seedling between 12 and 13 days old. I have found mycelium in the tip of the youngest leaf primordium to be cut off from the growing point in a seedling 20 days old, and in the growing point of a seedling 31 days old, and in both these cases the mycelium was intercellular. It was very much contorted, and stained with difficulty, thus differing markedly from the long intracellular hyphae found in the older tissues of the plant. Seedlings 38 and 40 days old (text fig. 21) showed mycelium plainly in the apex of the cone of the growing point. This mycelium was in isolated short fragments and appeared intercellular, the hyphae lying in the plane of the sections, and thereby appearing as if lying on top of the cells but not within them.

Below the growing point the hyphae were both intercellular and intra-

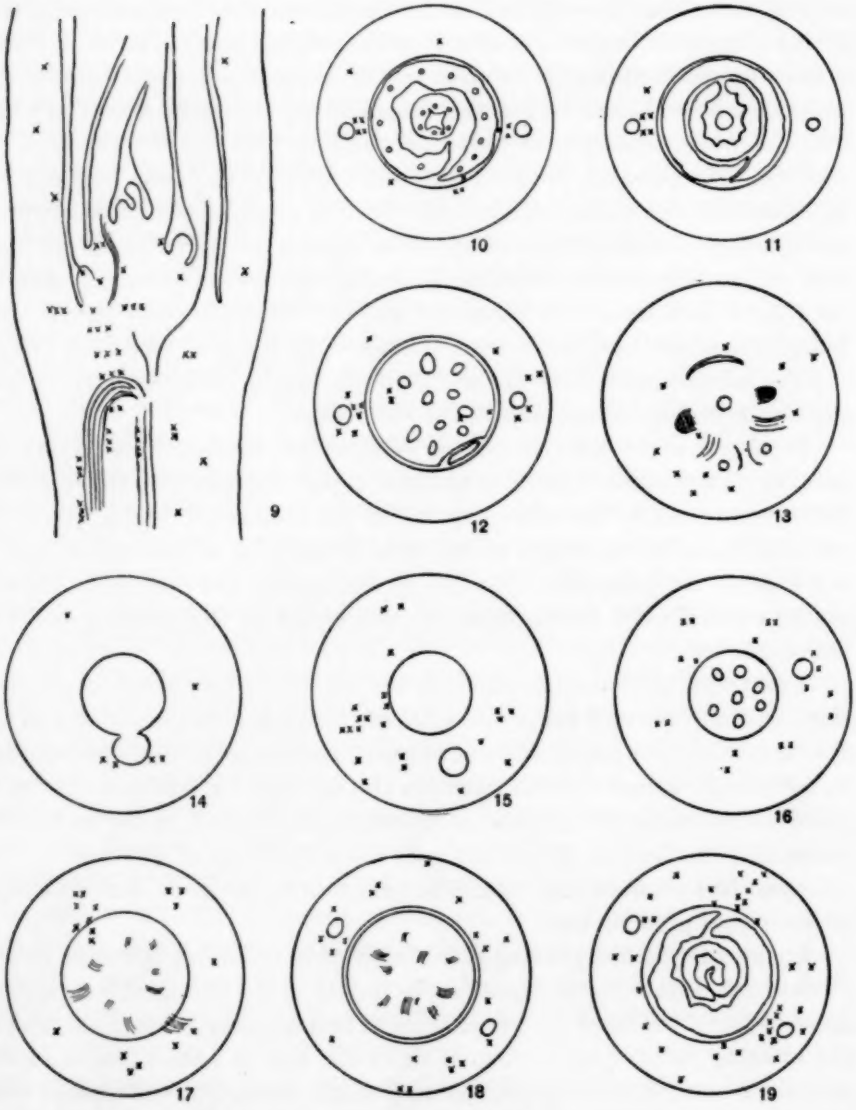


Fig. 9. Median longitudinal section through the same seedling as in figure 8. Figs. 10–15. Cross sections through a seedling five days old, above, through, and below the coleoptile node. Seedling germinated in sand. Figs. 16–19. Cross sections through a seedling eight days old, at the coleoptile node, and above it. Seedling germinated in sand.

cellular (text figs. 20, 21). This region consists of nodes closely crowded together. Up near the growing point, it was difficult to determine whether the hyphae were actually inside the cells, but in a seedling 26 days old a convincing case of cell penetration by a hypha was found about 0.4 mm. from the tip in the region of origin of the fourth and youngest leaf primordium, and in a seedling 40 days old, about 0.9 mm. from the tip, in the region of origin of the second oldest leaf. Nearer the coleoptile node, more undoubted cases where the hyphae were intracellular were found. When the internode between the coleoptile node and the first leaf node had begun to elongate, as was especially evident in the 38 and 40 day old seedlings, long intracellular hyphae were found in this region which frequently ran parallel to the long axis of the seedling through the cells adjoining the vessels, and even in the vessels themselves. These hyphae frequently showed collapsed and empty portions, a condition which will be described in more detail in the cytological part of this paper.

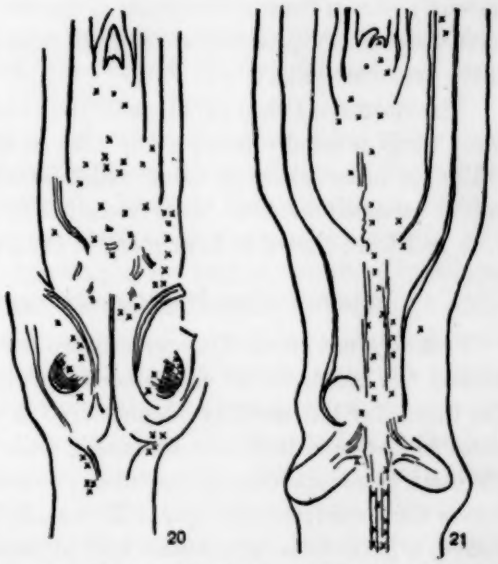


Fig. 20. Longitudinal section through a seedling 26 days old, showing region from growing point down to coleoptile node. Fig. 21. Median longitudinal section through a seedling 38 days old, showing region from growing point down to coleoptile node.

A study of the diagrams (figs. 1, 4, 5) shows that the mycelium in the early stages of development of the seedling is not found in the scutellum, in the root node, or lower portion of the mesocotyl. That found in the epiblast and mesocotyl of an eight day old seedling is too widespread to give any clue as to how and when it originally got into the seedling. Penetration through the coleoptile, both in the region of the coleoptile node, and in the upper portions of the coleoptile as shown in figures 2–5, even at the tip, is probably at least to be considered as an index of infection. It is found in the very early stages of development of the seedling, that is, in seedlings three and four days old. Moreover, the mycelium grows very quickly down along the inner epidermis toward the region of the growing point (fig 4.)

Effective penetration from the standpoint of infection probably also takes place in the mesocotyl right below the coleoptile node (figs. 6, 7). Seedlings five days old and older often contain an abundance of mycelium, the hyphae running parallel with the long axis of the seedling, growing upward and downward, and also extending radially inward from the outer cell layers of the seedling, both horizontally and obliquely. This mycelium apparently comes from germ tubes of spores, or more often sporidia, which have successfully penetrated the epidermis of the coleoptile or of the mesocotyl near the node.

The claims of Peklo (1913), and Jodidi and Peklo (1929) that symbiotic smut fungi normally inhabit the cells of the aleurone layer of the cereal grains, in order to be of value, must be substantiated by evidence of an actual establishment of these so-called symbionts in the growing point, such as I have shown to take place in the pathogenic oat smut, *U. Avenae*.

Cytological study of the relations of host and pathogen

Initial penetration. The germ tubes from conidia are capable of penetrating the coleoptile at any place from its tip to the coleoptile node. In the three day old seedling diagrammed in text figures 2 and 3, numerous holes in the cuticle of the coleoptile were noted (marked × in figures). Through these openings germ tubes proceed which can be traced directly across the epidermal cells (plate 27, figs. 1, 2). In one case (fig. 1), as noted above, a germ tube was found still attached to a chlamydospore, which was lying outside the epidermis, closely applied to the cuticle. This occurred right above the coleoptile node. In this case the penetrating tube, instead of arising from a conidium, was apparently produced directly from the chlamydospore without the previous formation of a promycelium and conidia. So far as I know, reports of direct infection of the host from a chlamydospore are rare in the literature of the smuts, except in the case of the loose smuts of wheat and barley. Lange (1910) has figured a promycelial tube making its way through the style of the wheat blossom, which was produced from a chlamydospore resting on the stigma, and Güssow and Conners (1927) reproduce this figure. The chlamydospores of seedling infecting smuts are generally thought necessarily to produce promycelia with conidia on germination. These conidia then are assumed to form germ tubes which gain entrance into the host. Tisdale and Tapke (1924) produced seedling infection of barley, however, with the flower-infecting *Ustilago nuda*, and figure germ hyphae proceeding directly from the spores, penetrating the coleoptile. However, Arland (1924) reports in inoculation experiments, that spores of *U. Avenae* germinating on the stigma of oat blossoms, sometimes form mycelium directly, and considers this to be due

to high temperature at the time of germination, and Jones (1923) found in germination tests of *U. Avenae* that spore germination took place up to 34° C., but no conidia were formed at temperatures higher than 30° C. The seedlings I have studied were germinated at room temperature (usually not more than 23° C.), but since the temperature was not controlled, it is possible that the lack of conidia production may be due to an increase in temperature not noted. Other seedlings germinated at different times and in a different place, but also at room temperature showed production of conidia, but the stages studied (one day old, 2½ day old seedlings) were too young to show penetration of the host.

The holes in the epidermis through which the germ tubes make their way into the seedling are very conspicuous (plate 27, figs. 1–4), and are strongly reminiscent of those figured by Brefeld. They are in each case the diameter of the hypha, and there is no indication of a narrowing of the tube in order to pass through a small opening in the wall as described by Blackman, Boyle, Dey, Waterhouse, and Brown for *Botrytis*, *Sclerotinia*, *Colletotrichum*, and sporidia of *Puccinia graminis*. The cuticle takes a yellowish brown stain with the orange of the triple stain, whereas the hypha takes a reddish-blue stain. The hole is represented by a clear colorless space in the cuticle or epidermis. In figures 3 and 4 (plate 27) the position of the hole indicates that the germ tube penetrated somewhat obliquely from a plane slightly above that of the drawing. Tisdale (1924) has figured holes caused by germ tubes of *Ustilago nuda* in barley seedlings, very similar to those of Brefeld's. He makes no mention of the method of penetration, but in another paper, on flax wilt (1917), he states that the diameter of the openings made by a hypha is somewhat less than the regular diameter of the hypha.

The germ tube, after penetrating the cuticle and the subcuticular layers of the epidermal cell wall, reaches the plasma membrane. Figures 1–5 (plate 27) are drawings of epidermal cells into which the germ tubes have recently penetrated. A striking characteristic of these entering germ tubes noted in all cases of penetration is their direct course through the cell, some times obliquely, but most often at right angles to the long axis of the cell. The cytoplasm of the host cell in these cases is vacuolated, but the single large central vacuole, as it is found in older cells (fig. 6) is not yet formed. Whether actual penetration of the plasma membrane or merely invagination takes place, cannot be ascertained from figures 1–4, but figure 5, illustrating a case of a very much swollen germ tube, gives the appearance of invagination. The shrinkage of the cell contents away from the cell wall and from the hypha, accentuates this appearance. In these three day old seedlings, as has been stated, there is no large central vacuole. Therefore,

the developing hypha, as shown in figures 1–5, grows straight across through the cytoplasm of the cell, perhaps invaginating the plasma membrane, perhaps not. The important thing, however, brought out by a comparison of figure 6 with figures 1–5, is that in both cases the fungus hypha remains embedded in the cytoplasm of the host cell. In the case of figure 6, a five day old seedling in which the epidermal cells have large central vacuoles, the hypha lies in the primordial utricle close to the cell wall. There is no attempt on the part of the hypha to puncture the tonoplast in any of the three cells pictured, and make its way into the central vacuole. This was also noted in other cells and will be referred to later. In the case of the young seedlings, however, the course of the hypha is not limited to the peripheral region inside the cell wall, but may follow any direction that the strands of cytoplasm take.

The binucleate condition of the cells shown in figures 1 and 2 was observed many times in these entering germ tubes. In view of the findings of a similar condition by Rawitscher (1912) in the tissues of the young oat blossom, and his conclusion, although unwarranted from his material, that the binucleate condition arises from fused conidia or fused promycelial cells, these cases are suggestive. A careful study was made, not only of hyphae in the epidermal cells, but also of hyphae in all the tissues of the seedlings, in respect to the number of nuclei per cell. The cells of the hyphae are relatively long and it is not easy to find sections showing the full length of a hyphal cell with both septa well marked. Hyphae were studied which were located in the outer and inner epidermis of the coleoptile, the parenchyma cells of the coleoptile, the space between the coleoptile and the first leaf, in the cortical parenchyma and central stele of the mesocotyl (in older seedlings), in the tissue of the node, and in the tissue below the growing point. Sixty-two cases were found in which the portion of the hypha showed one nucleus; 107 cases in which two nuclei were undoubtedly present, and eight additional cases which were questionable. More than two nuclei were found in nine cases, seven of which had definitely three nuclei, and five additional cases which were questionably multinucleate. The small size of the nuclei in the oat smut, the length of the hyphal cells, and their frequently twisted and winding form, contribute to the difficulties of the problem. However, wherever possible small branches including tips of hyphae, or portions of hyphae between definite septa were studied, or else hyphae without septation but sufficiently long to give a fair estimate of whether or not more than one nucleus might have been present. Almost twice as many cases of a binucleate condition were noted as a uninucleated condition (107 to 62) and very few cases of multinucleate condition (9, possibly 14). However, the figures show that enough unquestionable cases

of each type were found to indicate that the cells of the mycelium of the oat smut in its early stages of parasitism may be uninucleate, binucleate, or multinucleate. In the outer epidermal cells no multinucleate hyphae were found, except a questionable case in which four nuclei were indicated. Figure 18 (plate 30) shows an undoubted case of the multinucleate condition in a hypha in the cells of the inner epidermis of the coleoptile; figure 16 (plate 29), a decided binucleate condition, in a hypha in the cells of the cortical parenchyma of the mesocotyl. In six cases adjacent cells in the same hypha differed, one cell being uninucleate, the next binucleate. In one case the swollen tip of the hypha contained three nuclei, while each of the two cells behind this contained a single nucleus. It was not at all unusual to find a single nucleus in the short stubby branches characteristic of smut mycelium, and frequently adjacent cells in a hypha were uninucleate. Only in the nine cases referred to as questionably binucleate was there a question of whether or not a wall was present between the two nuclei.

Figure 5 (plate 27), already referred to, shows a case of a degenerating infecting germ tube, probably from a conidium. It was found near the tip of the three day old seedling illustrated in text figure 5. The hole in the cuticle by which the fungus entered is indicated. The whole structure is much thicker than the normal hyphae shown in figures 1–4, and is funnel-shaped. The wall stained a light blue color similar to that of the subcuticular layers of the epidermal wall of the host cell, but whether this can be taken to indicate that a cellulose sheath has surrounded the hypha and interfered with its growth, as described by the older writers, e.g. Fischer von Waldheim, and Wolff, is open to question. Certainly there is a close continuity of the thickened envelope of the hypha with the subcuticular layers of the host cell. A comparison of figures 1 and 5 shows without question that the latter does not represent a normal healthy hypha. In the specimen, no cytoplasm can be seen through the thickened wall, and there is just a suggestion of contents, indicated in the drawing by a fine line, where the hypha thins out toward the inner wall of the epidermal cell. Brefeld observed such cases and explained them as due to degeneration. These are hyphae which have penetrated to a certain distance and have then been stopped. A further discussion of this point will be taken up in connection with similar phenomena in older hyphae which have penetrated further into the host.

Penetration from cell to cell. Once inside the host, the hypha makes its way intracellularly through the parenchyma cells. These hyphae are either binucleate or uninucleate. Multinucleate hyphae with the exception of the one figured in the inner epidermis of the coleoptile in figure 18, which was found in a four day old seedling, were usually found in older seedlings, in

the parenchyma of the coleoptile, or in the cortical parenchyma of the mesocotyl. As the hypha reaches a cell wall, it forms a spherical tip, closely appressed to the cell wall. This stage was observed at least a dozen times in five day, eight day, and thirteen day old seedlings and is shown in figure 7 (plate 28) although here it is not as pronounced as in some other cases. In several instances a point on the opposite side of the host cell wall adjacent to the end of the hypha, showed a deep reddish stain (the safranin of the triple stain). This was noted in both the five and the eight day old seedlings.

There is no indication that the hypha penetrates as a fine process or thread. There is a distinct widening at the point of penetration, as though the rounded tip before mentioned had pushed its way bluntly through a softened wall. This is shown in figures 8, 9, 10, 12 (plate 28) and figure 11 (plate 27). Further indication that a chemical change may have taken place in the host cell wall is its reaction to the stain on either side of the hypha at the place of penetration. In the above mentioned cases, the walls of these parenchyma cells of the host are very thin, and stain a pale yellow with the triple stain, but around the hypha where it comes in contact with the host cell wall, there is a distinct reaction to the safranin stain. This reddening was generally present in all my sections, and I regard it as characteristic for this mycelium. The hypha is always thicker at the point of penetration; after passing through the wall it narrows abruptly to its original diameter before penetration.

By focussing up and down, the entire periphery of the hole, through which the hypha penetrates, can be studied, as shown in figures 8 and 12: figure 12 shows the change in staining reaction at the point of contact extending as a ring around the hypha, and this is also prominently indicated in figure 13 (plate 29).

Intracellular branching of the hyphae is common, as shown in figure 12. In figure 11, one of these short branches is shown originating at an intercellular space.

As the hyphae make their way through the parenchyma cells, they follow the distribution of the host cytoplasm, and are surrounded by it to a greater or less extent. Sometimes the layer of cytoplasm is very thin as shown in figure 13, and cannot be observed at all around portions of the hypha with little or no contents. At this stage figured from an eight day old seedling, the parenchyma cells have large central vacuoles, and several of my figures besides figure 13 (figs. 12 and 21) show a hypha apparently lying in the central vacuole, but nevertheless showing unmistakable evidence of cytoplasm surrounding it. In other cases, such as figures 14 and 15, there is shown a dense accumulation of host cytoplasm

around the tip of the hypha, and figure 16 (plate 29) shows dense cytoplasm enveloping the entire hypha as it makes its way through the cell. The tip has taken a more intense stain than the rest of the hypha, as shown in figures 14 and 15. Allen noted this massing of host cytoplasm around rust haustoria, and, in her earlier papers at least, considered it as a normal relation of host and parasite. Later she was inclined to consider it as a host reaction implying resistance, and leading to the formation of a definite covering or sheath around the haustorium. Guttenberg held this view also in relation to the intracellular hyphae of the corn smut. I have found that where the hyphae are surrounded by this mass of host cytoplasm, they appear particularly vigorous as judged by the staining of their cytoplasm and nuclei (figs. 10, 15, and 16). The variety of oats on which this study was made is particularly susceptible to the strain of smut used, and the massing of cytoplasm on the hyphae may be associated with this fact.

Course of the hyphae through the cells. The hyphae grow in all directions when inside the host, making their way up and down and across the parenchyma cells of the coleoptile, into the tissues of the node, and later into the parenchyma and stele of the mesocotyl. Frequently they are very long. In a cross section through the mesocotyl of a five day old seedling one hypha was noted running through five parenchyma cells (fig. 17, plate 29), a distance of 174.6 microns. Another hypha in a thirteen day old seedling, seen in tangential view, in a longitudinal section of the coleoptile, extended diagonally through seven parenchyma cells, a distance of 256 microns. This was probably not far from the point of penetration, but the exact point could not be ascertained.

The long section of hypha illustrated in fig. 17 shows striking differences in diameter and staining, which apparently are not due to methods of fixation and staining. The tip of the hypha at *a* stained densely, and also the portion of the hypha in the host cell from which it has just emerged. Back of this, at *b* is a very thin portion stained deeply violet, appearing in my drawing as scarcely more than a line. The third segment of the hypha *c* is broader than *b*, almost transparent, and merely tinged with violet, indicating very little cell contents. The portion *e*, running through two host cells, is broader than any other portion of the hypha, shows abundant cytoplasmic content stained pale violet, and contains two nuclei, lying close together but not in the same plane, so that only one is visible in the drawing. This portion of the hypha is cut off from the segment *c* by a distinct septum, right behind which a side branch *d* has developed which grows obliquely outward and extends in a plane slightly above that of the main hypha. This branch is also stained pale violet. At the other end of the segment *e*, at the point *f*, another branch has arisen which extends verti-

cally upward, and can barely be seen at the magnification at which this drawing is made. Behind the point of origin of this branch, there is an abrupt loss of staining capacity in the segment of the hypha designated as *g*. Very little cytoplasm is visible in this portion, and the wall of the hypha stains pale blue. This portion is less in diameter than segment *e*. The characteristic reddish stain (safranin) where the hypha penetrates a host cell wall can be noted particularly at two points in the course of this hypha. As shown in the drawing, the hypha throughout its entire length was invested with host cytoplasm, whose outlines could be seen with little difficulty. There is a notable tendency on the part of this hypha to follow the walls of the host cell. This may be due to the fact that the parenchyma cells of the mesocotyl at this stage of development have large central vacuoles and very thin primordial utricles. The portions *b*, *c*, and *g* probably represent degenerating segments. Portion *e* is still fairly well nourished and shows two branches, but the rest of the hypha shows various abnormal appearances indicative of degeneration.

The reduction of the hypha to a mere strand of violet-stained material (at *b*, fig. 17) probably indicates degeneration of another type, and may be similar, although staining differently, to that which will presently be described for figure 13, *c* (plate 29) and figure 18 (plate 30). Empty portions of hyphae are also illustrated in figure 13 at *b*, *c*, and *d*, and figure 16 at *a* (plate 29) and were seen many times in both fixed and fresh material. In the literature, empty portions of germ tubes and promycelial hyphae have been frequently figured in smuts growing in culture media. Brefeld (1883, 1895) calls this phenomenon 'Kammerung,' and figures it for practically all the smuts cultured by him. Lutman (1910) figures it in the promycelial cells of *Ustilago Hordei* (fig. 30), and in old germ tubes from conidia of *U. levis* (fig. 3). Osner (1916) figures empty portions of germ tubes in *U. striaeformis* (fig. 54). According to these authors, these empty portions occur either at the proximal end of the germ tube near the spore, or may be intercalary; they sometimes also appear in one of the two fusing promycelial cells (Lutman, fig. 30). Inside the host, Brefeld also describes them as occurring in the germ tube near the point of penetration. These cases illustrate the more or less general idea in the literature on smuts and rusts, that at least under certain conditions, the living contents of the germ hypha advance with its elongation leaving very little behind. Figure 3 (plate 27) shows a condition similar to that described by Brefeld, in which the cytoplasm of the entering germ tube has advanced toward the tip of the hypha, and left an empty portion cut off by a septum at the point of penetration. Owing to the minute size of the object, it is impossible to tell whether or not a thin layer of cytoplasm might not be lining the supposedly empty portion of the tube.

Less familiar than these less stainable but still apparently turgid hyphal segments, are those which are reduced to merely filiform strands (fig. 13, *c*, fig. 18). The walls of the hyphae may still be distinctly differentiated, taking a brownish yellow tinge due to the orange G of the triple stain. The condition of the hypha as shown in figure 17 at *b* is similar, with the exception that it is stained distinctly violet, and the wall cannot be made out clearly. How this filiform condition of the hypha is brought about is not easy to determine. It is possible that more or less empty portions of the hypha may have collapsed. Figure 13, *c* (plate 29) in my drawings perhaps might be interpreted in this way, especially because of its trumpet-like widening to the normal diameter at the point of penetration of the cell wall. Rawitscher (1912) figured collapsed promycelial cells in *Ustilago Carbo* in culture media; Osner (1916) has described collapsed portions of germ tubes of timothy smut, also in culture media. Roesch (1926) has figured what he regards as long, drawn out hyphae in coleoptile tissue of oat seedlings, and describes the hyphal walls as 'zusammengedrückt.' Often these narrowed segments of the hyphae have the appearance of being drawn out into a long strand, due to stretching. In fresh material I have found such apparently long drawn out strands in the cells of the inner epidermis of the coleoptile, when I studied this tissue stripped off in thin ribbons. I have also found them in fixed material in both cross and longitudinal sections, and in median and tangential views of the cells as shown in figures 18, 19, and 20. Brefeld, who also observed this condition of the hyphae, offers two different explanations for it. In oats, he considers it due to inability of the fungus to keep pace with the growth of the host cell, resulting in a stretching of the hypha as the host cell elongates. In the cases in corn, where he stripped off the epidermis of the leaf sheath for study, he attributes it to the method of preparation of the material for observation. Brefeld's second explanation could hardly apply in the case of fixed material. A portion of a hypha from a four day old seedling is shown in figure 18 (plate 30), as seen in tangential view. It extended through five cells of the inner epidermis of the coleoptile, at right angles to the long axis of the seedling, and was 97 microns long. The characteristic widening of the hypha at the points where it penetrates the cell wall is conspicuous, as well as extreme narrowing in the middle region of the host cell. I have seen similar conditions in fresh material. Figure 19 shows an apparently stretched portion of a hypha in a parenchyma cell at the base of the coleoptile node. The finely attenuated end apparently disappears in the host cytoplasm. Figures 20 and 21 also show hyphae whose form suggests stretching. Figure 20 is part of a hypha which I traced through a length of 354.05 microns extending through five parenchyma cells of the mesocotyl parallel to the long axis of the seedling.

There is need of further evidence as to the cause of this apparently stretched condition of the hyphae. Brefeld's assumption that the host cells stretch after the fungus has entered them is plausible, but we need to know more specifically as to the time and place of penetration of the smut hyphae, and the time and place of elongation of the various parts of the oat seedling. Bauman (1910) has attempted to determine more exactly the regions of elongation in the coleoptiles of the different grains, and he found that in wheat, rye, and barley, growth is basal as long as the sheath is very short, but soon advances to the tip. In oats, however, he found the course of growth of the coleoptile different according to varieties and external conditions. Basal stretching was pronounced, as well as apical stretching. I have measured the lengths of the parenchyma cells in the coleoptile and mesocotyl at successive stages of seedling development. From eight to fifteen parenchyma cells in longitudinal sections of the coleoptile and mesocotyl were measured by means of the eyepiece micrometer, and their average length determined. The cells were chosen so as to represent both extremities and the middle of both organs. Cells of the coleoptile of seedlings two, three, four, and five days old were studied, and of the mesocotyl, also cells of the latter organ in a 13 and a 20 day old seedling. The two day and four day old seedlings were from the same planting, the five day old seedlings were from another planting, the three day old seedlings from still another planting. The results were as shown in table 3. These figures take no account of the rate and time of cell division.

TABLE 3

Average length of cells of coleoptile and of mesocotyl

SEEDLING AGE	2 DAYS	3 DAYS	4 DAYS	5 DAYS	5 DAYS	13 DAYS	20 DAYS
coleoptile	45.25μ	32.8μ	77.5μ	188μ	115μ		
mesocotyl	103.3μ	63μ	186.2μ	147.2μ		86.2μ	156.9μ

Although the cells of the coleoptile seem to show a progressive increase in length with the age of the seedling (except those of the three day old seedling), those of the mesocotyl do not show such a decided increase. The latter condition may be due to the fact that most of the growth in length of the mesocotyl takes place before the seedlings are above the ground, i.e. before they are four days old. Although not conclusive, I think there is evidence here for increase in length of the cells at the time when the organ is externally giving evidence of growth in length. Hyphae, then, that have gained entrance into the coleoptile or reached the mesocotyl cells in the

vicinity of the node while this stretching is going on, might well become stretched, as Brefeld suggests. I have not seen dividing cells with hyphae in them. The possibility that these hyphae may appear stretched, because they have lost their contents and then shrunk is not to be disregarded.

Figures 20 and 21 from a 20 day old seedling illustrate a condition in these apparently stretched hyphae which has been especially observed in older seedlings (13 and 20 days old). The hypha shows a varying thickness, takes very little stain, and is more or less translucent. In its axis there is seen a rather discontinuous, thread-like line of pale purplish stained material (fig. 21). As shown in figure 20, such a hypha may suddenly swell out into a thickened bulbous portion which stains very lightly. At the ends of this bulbous portion, the axial strand of purplish staining material widens out so as to fill the hypha. In these points my preparations show conditions strikingly like some of those of Guttenberg. Guttenberg assumes a stretching of the hypha inside a cellulose sheath, formed by the host cell, primarily to prevent the parasite from establishing food relations with the host, but serving also to prevent the hyphal strand from tearing apart, and so disturbing the tension inside the cell. As noted above, the formation of thickened sheaths around a hypha or haustorium has been described many times in the literature of the smuts and rusts (Fischer von Waldheim, Wolff, Lutman, Dastur 1920, Allen, Rice, Ruttle, and Fraser), and all of these authors consider such sheaths as host reactions to the parasite. The possibility that the thickening may be due simply to a swelling or gelatinization of the hyphal wall in process of degeneration, is however not excluded. In the fungi, swollen germ tubes are described by Brefeld, as before noted, and Allen describes swollen haustorium mother cell walls in the rusts, but she considers this as an indication of resistance on the part of the host. My figure 9 (plate 27) shows a thickened hypha with the lumen almost occluded. This, together with figures 20 and 21, may be interpreted as indicating swelling of the hyphal wall prior to disintegration. As shown in figure 20, the swollen portion of the hypha perhaps marks the point where it is about to break. Brefeld suggests that such isolated fragments of mycelium, with still some living portions, as figure 18 and 13*a*, which have been unable to reach the growing point, may persist in the tissues near the node, where they may later infect the buds of the secondary shoots or tillers.

Intercellular hyphae in the coleoptile and mesocotyl near the node are also to be found. In figure 6 (plate 27) the tip of the hypha appears to be making its way between two of the epidermal cells, and in figure 11, an intercellular branch is arising. Frequently cross sections of hyphae were noted in the intercellular spaces. Hyphae were also noted inside the lumen

of vessels, and abundant mycelium was found in the space between the coleoptile and the first leaf (fig. 22). Mycelium in adjacent regions of the inner epidermis of the coleoptile and the leaf indicates that crossing over this space is not uncommon, and takes place both near the coleoptile tip and near the node.

I regard all the hyphae shown in figures 13, 16–21 as normal phases in the development of the hyphae as they grow toward the apical meristem. Even in the tissues below the growing point, these long intracellular hyphae, with portions showing these various appearances of degeneration are found without difficulty. In the growing point, however, they are no longer to be found. Here the hyphae appear as short, curiously curved segments, with both ends plainly visible (fig. 23). The curvature of the hypha seems to follow the contour of the host cells, which however are in no way distorted by the presence of the pathogen, and as to their content, appear like those of any normal growing point. The large host cell nuclei are especially well stained. I consider the fungus as intercellular, and in my drawing have endeavored to show that it is lying on top of the host cells, in the plane of the paper. Nuclei are visible in the hypha, but they stained very faintly and the exact number could not be ascertained. This hypha was located in the apex of the cone of the growing point of a seedling 38 days old. Hyphae in the growing point were also seen in a seedling 31 days old, and in one 40 days old. Sections through a very young panicle showed hyphae in the various primordia very similar to those described in the vegetative cone of the 38 and 40 day old seedlings.

The course of development of the fungus inside the host may be summarized as follows:—A germ tube produced from a conidium or a chlamydospore penetrates the cuticle and makes its way through the epidermal cells of the host. This germ tube may be uninucleate or binucleate. I have no further evidence as to whether the binucleate condition arises by division of a single nucleus, or whether the nuclei come from a pair of conidia. I find no indication of conjugate nuclear division, and the presence of cells with three nuclei is against its regular occurrence. Within 30 days, the hyphae make their way through the parenchyma cells of the coleoptile, and cross the space between the coleoptile and the first leaf. The ability of the hyphae to ramify in this space has been amply demonstrated (text figs. 4 and 5, fig. 22, plate 30). In a seedling four days old whose coleoptile measured 1.5 cm., the hyphae between the coleoptile and the first leaf had made their way half the distance from the tip of the coleoptile to the coleoptile node (text fig. 4, above referred to). They then penetrate through the tightly rolled leaves to the growing region. There is also a wide ramification of the hyphae in the tissues of the coleoptile as the seedling grows

older. They may grow upwards toward the tip, or downward into the tissue of the mesocotyl. Hyphae in the inner epidermis of the coleoptile may also make their way into the parenchyma of the coleoptile toward the outer epidermis.

The course of the hyphae through the host cells is dependent on the distribution of their cytoplasmic contents. Where the host cell has dense cytoplasm or strands of cytoplasm crossing the cell, the hypha grows directly through the cell, and may perhaps stimulate the host cell to produce more cytoplasm. Where the host cell has a large central vacuole, the hypha tends to follow the thin layer of cytoplasm inside the wall. The older portions far removed from the tips of the hyphae show degeneration changes and become practically empty of contents. Stretching of these empty portions due to growth of the host cells and collapse of the empty walls may occur, giving to the hypha the appearance of filiform strands, which however still show the characteristic widening at points of penetration of the host cell walls. Portions of these older hyphae, still in the living condition, are left isolated in the tissue of the host and may be a source of tiller infection.

In the cone of the growing point the hyphae become intercellular and are not conspicuous, but in the tissues of the growing region below the growing point there is abundant mycelium, both inter- and intracellular, and showing the characteristic degenerating hyphae. Microtome sections through the growing tip of the seedling show only very short hyphal fragments between the cells.

The susceptibility of this variety of oats (Victor 126) apparently depends on the ability of the fungus to reach this apical region of the host. A study of an immune variety of oats, Black Mesdag, made along with these studies on Victor, failed to show any mycelium in the growing point or adjacent tissues in seedlings 7, 10, and 15 days old. In serial sections of these seedlings cut 15 microns thick, hyphae were found only rarely, and then only in coleoptile tissue. The most mycelium was found in a 10 day old seedling up near the tip in the coleoptile, sixteen sections out of a total of 360 sections showing the presence of a hypha. No mycelium was found in a seedling 10 days old in the coleoptile near the node, the node itself, or in the mesocotyl immediately below the node. Only two sections out of 270 showed a hypha in the 7 day old seedling, and only two sections out of 144 showed a hypha in the 15 day old seedling.

DISCUSSION

The evidence from infection experiments brought by Dickinson, Stakman and Christensen, and Hanna, which tends to prove the existence of

heterothallism in smuts, carries further the work of Kniep (1919, 1921, 1926, 1928, 1929), who, with the aid of Bauch (1922, 1923, 1925), has done much to establish the old De Bary idea (1884) of the sexual significance of conidial fusions in the smuts. The older literature on this subject has been thoroughly reviewed by Blizzard (1926). It is still not clear how long conidial cultures can be continued without fusions, nor is the method of germination and the possibility of further continuing the culture after fusion adequately worked out. It is a generally accepted view that reduction divisions occur when the smut spore germinates, and according to the sex chromosome theory, at this division the sex of the succeeding cell generations is determined. The propagation by budding which may follow is then gametophytic. If the promycelial cells and conidia are gametes, as is suggested by the tendency they frequently show to fuse immediately, the plus and minus strains which are produced by their budding must be regarded as propagated by parthenogenesis and androgenesis respectively. The fact that cell fusion may be delayed through a longer or shorter period until apparently sexual maturity is reached, suggests that they are rather to be regarded as gametophytic haploid spores whose budding constitutes a longer or shorter haplontic generation. It is now being generally assumed (Rawitscher, Kniep, Bauch, Seyfert, Stakman and Christensen, Hanna, Goldschmidt, Dickinson) that cell fusion results in a binucleate dikaryotic stage which continues till nuclear fusion occurs in the smut spore. But evidence as to the origin and length of this binucleate cell stage in the smuts is still not very clear.

The situation differs in the Ustilaginaceae and the Tilletiaceae:

Ustilaginaceae. Dangeard (1892) describes the sporidia of the Ustilagineae as uninucleate and this has been confirmed by Harper, Rawitscher and others. According to Lutman (1910), the number of nuclei in the conidia of *Ustilago levis* varies according to the medium in which they are grown. In liquid rich in nutrients, there was abundant reproduction by budding, and these conidia were uninucleate. On agar plates, the conidia lost their oval shape, became swollen and irregular, and contained two or more nuclei, often four or five. In host tissue, Lutman observed a multinucleate condition of the cells in the young hyphae of *U. levis* five days after oat seedlings were infected, and reports that *U. Zeae* also forms a parasitic mycelium with multinucleate cells. According to Kniep in *U. violacea* (1919), and Bauch in *U. longissima* and its variety *macrospora* (1923), the conidial fusions initiate the binucleate condition which is continued in the host plant with the beginning of parasitism, and is carried on by conjugate nuclear division until the nuclei fuse in the spore. However, neither Kniep nor Bauch made a study of the mycelium in the host

plant, so that this sequence stated by Bauch to be the normal type of development for the Ustilagineae (Bauch 1923), and now generally accepted, is still to be proved by actual observations. Rawitscher (1912) offered some evidence for the binucleate condition in the parasitic mycelium of *Ustilago Carbo* on oats, but he describes this only for the young floral parts, and apparently made no study of the nuclear condition of the smut in the young seedling, although he states that in *U. Carbo* we have a smut form which goes through the largest part of its development in the binucleate stage. Rawitscher only figures a hypha (binucleate) penetrating the epidermis of a young floral part (Blütenteil). His claims as to the origin of the binucleate cell stage in *U. Zeae* by hyphal fusions at the time of spore formation have been questioned in the recent work of Seyfert (1927), who claims to have found the binucleate condition long before spore formation. Hanna's claims (1929) that the germ tubes of *U. Zeae* fuse shortly after penetration of the host plant certainly need further confirmation.

Tilletiaceae. Dangeard (1892) shows the secondary sporidia which give rise to the mycelium as binucleate, due either to a bipartition of the nucleus of the primary sporidium (*Urocystis*, plate 22, figs. 10–12), or to conjugation of the primary sporidia in pairs (*Tilletia*, plate 23, figs. 15–18). Lutman found the cells of the mycelium binucleate in *Doassansia*, *Urocystis* and *Entyloma* in the host tissue. Rawitscher (1914, 1922), Paravicini (1917), Kniep (1921) made a study of sporidial and promycelial fusions as a basis for their claims that these fusions give rise to the binucleate condition, but here as in the Ustilagineae, evidence of the binucleate condition in the young penetrating mycelium is either wholly lacking or not convincing. Dastur (1921) studied the conjugation of the primary sporidia of *Tilletia Tritici*, and describes the passage of the nucleus and cytoplasm of one sporidium into the other, which then develops a secondary sporidium, usually uninucleate, but sometimes binucleate. A fusion of 'conjugate' nuclei is described in the secondary sporidium. The presence of two nuclei in the secondary sporidium is claimed to be due in some cases, at least, to the division of a single non-conjugate nucleus. In the early stages of infection, the hyphae are described as uninucleate or multinucleate, but not binucleate. The diversity of these findings leaves the problem as to the origin and nature of the binucleate condition still unsettled. Sartoris (1924) claims that the germ tube arising from fused sporidia of *Tilletia Tritici* penetrates the epidermis of the wheat seedling, while Noble (1924) claims that in *Urocystis Tritici* the germ tube which penetrates the epidermis of the coleoptile arises from a single sporidium, and not from conjugated sporidia. Blizzard (1926) obtained uninucleate saprophytic mycelium without conidia in cultures of *Urocystis Cepulae*, and succeeded in infecting

onion seedlings with it. This parasitic mycelium was at first uninucleate, but became progressively binucleate as it approached the young sorus primordium.

Although the evidence for the origin of the binucleate condition in both groups of the smuts (the Ustilaginaceae and the Tilletiaceae) shows that various and contradictory opinions exist, yet it has come to be established more or less as a fact that the young spores in the smuts are binucleate, and on maturing become uninucleate by fusion (Dangeard, 1892; Harper, 1899; Lutman, 1910; Rawitscher, 1912, 1922; and others).

In view of this situation, Boss's findings (1927) are very interesting. He studied *Ustilago Ischaemi*, *U. hypodytes*, *U. perennans*, *U. violacea*, and *Tilletia Tritici* both in culture media and in the host. In *U. Ischaemi*, in culture he finds that even the young spore is not binucleate. It is uninucleate from the beginning, and is formed by the septation of a multinucleate mycelial hypha. In the host, he finds only uninucleate spores. He also describes the formation in culture of uninucleate chlamydospores in *Tilletia Tritici*.

Boss also reports that in *U. violacea* the secondary sporidia arising from fused sporidia show only one nucleus, as did also secondary sporidia of *Tilletia Tritici*. This he interprets as a breaking up of the binucleate condition, thereby invalidating any assumption of sexual significance in the fusion.

Boss's uninucleate chlamydospores in the smuts may be taken as evidence for the ability of a smut fungus to carry through its life cycle in the haploid condition. Dodge's uninucleate aecidiospores in the short cycled *Caeoma nitens* illustrate a like case in the rusts. Other cases are Moreau's *Endophyllum Euphorbiae* with uninucleate aecidiospores, and Bauch's *Camarophyllus virgineus* (1926), in which there is no nuclear fusion in the two-spored basidium. This condition in the Basidiomycetes, of a uninucleate basidium producing two spores without a nuclear fusion and without a reduction division preceding spore formation, has been described by Maire in 1901 and 1902 for *Hygrocybe conica* and *H. ceracea*, and confirmed in 1911 for *H. conica* by Fries.

Sass (1929) reports two-spored forms of *Coprinus ephemerus*, *Naucoria semiorbicularis*, and *Galera tenera*, in which four-spored forms are already known. Sass does not figure any hyphal fusions. He finds nuclear fusions in the basidia of these two-spored forms.

In four-spored forms, haploid fruit bodies arising from monospore cultures have been reported in a number of heterothallic strains (Bauch, 1926, p. 368), but cytological investigation has not yet established the fact that they produce uninucleate basidia without karyogamy. The single cytological investigation of a four-spored form producing uninucleate basidia is

that of Kniep (1911) for *Armillaria mellea*, but these are basidia borne on the mycelium, whereas the four-spored basidia produced on the gills of the fruiting caps show two primary nuclei which fuse.

In *Coprinus stercorarius*, and *C. narcoticus* reported by Brunswik (1924) the spore is binucleate, and although clamp connections appear very soon after the spore germinates, the cells are multinucleate, and the multinucleate condition continues in the fruit body up to the trama of the lamellae, and only the hymenial elements are binucleate. According to this evidence, the importance attached to clamp connections by Bensaude and the school of Kniep (i.e. that they serve to maintain the dikaryotic condition in the secondary mycelium) may be questioned.

In the literature, reports on the presence of clamp connections, even in the same form vary widely. Brunswik reports no clamp connections in the mycelium of the homothallic *Coprinus ephermeroides*, and none in the heterothallic *C. ephemerus*, *C. curtus*, or *C. radians*. In *C. ephemerus* he fully confirms Brefeld on this point, since no clamps were found in mycelium, aerial mycelium, in fruit body tissue, or in regeneration from different parts of the fruit body and the individual lamellae. But Sass reports clamp connections in secondary mycelium of both the two-spored and four-spored forms of *C. ephemerus*, although he states that clamp connections were not found in the subhymenial trama, and never at the base of a basidium in either *C. ephemerus*, *Naucoria semiorbicularis*, *Galera tenera*, or *Psalliota campestris*. Sass stresses the lack of clamp connections in the pileus of forms having a pseudo-parenchymatous trama, and the presence of clamp connections in forms in which this tissue is loosely filamentous (*Hypochnus sublitis*, observed by Harper, *Typhula* observed by the author himself, and *Armillaria mucida* observed by Kniep).

Sass's view is that the large pseudo-parenchyma cells can accommodate the two conjugate spindles without the necessity of clamps. Brunswik finds the clamps difficult to explain, either as a mode of maintaining the paired nuclei or as a means of facilitating transportation of food materials. In the former case, the many Hymenomycetes without clamps seem to have maintained the binucleate condition by cell division in a much simpler way, and in the second place, these 'clamp-less' Hymenomycetes often form the most luxuriant mycelium. He is inclined to regard the clamps as phylogenetic heirlooms.

We have seen that in the smuts (*Ustilago Ischaemi*, *Tilletia Tritici*), in the rusts (*Caeoma nitens*, *Endophyllum Euphorbiae*), in the Hymenomycetes, the presence and fusion of two nuclei is not necessary in all cases for the formation of a chlamydospore, an aecidiospore, or a basidium; haploid development is quite a common occurrence. Moreover, in the light of Bruns-

wik's evidence from the Hymenomycetes, we see that the binucleate condition is not a fixed condition originating at a definite time in the life history and continuously maintained by clamp formation; that frequently there are no clamp connections at all; that fruit bodies occur nevertheless; and that even on clamp bearing mycelia the production of fruit bodies varies in different pairings of plus and minus mycelia, some pairings producing fruit bodies abundantly and quickly, others producing none, or only a few after a lapse of time and frequently without spores.

However, Kniep's work (1919, 1921, 1926) on the sexual significance of conidial fusions seems to be more or less generally accepted, and the opposing view as expressed by Brefeld (1883) and by Harper (1899) that the conidial fusions are merely vegetative, has not been supported by later workers. Kniep's view is based on culture work with *Ustilago violacea*, in which he obtained single sporidial cultures by the poured plate method. He found that sporidia from such a culture do not fuse with each other, and that sporidia from different cultures when brought together show conjugation in about half of the cases. This he interprets as indicating that the sporidia on a promycelium are of two different kinds physiologically, and that conjugation takes place only when unlike conidia are brought together. Bauch (1922) reports evidence that the old Brefeld idea that the conidia fuse because of exhaustion of food supply was erroneous. He inoculated old culture media, in which conidia had been growing, with fresh conidia after sterilization of the old cultures, and reports that only a few fusions were noted. He finds that the condition most favorable for conjugation is one which favors an intensive aeration such as is afforded in thin liquid layers, but all these experiments were made rather to determine the conditions favorable to fusion when both types of sporidia are present, than to determine if it might not be possible to get fusions with only one type of sporidium present. It is interesting to note that Laibach (1928) finds these same cultural conditions that favor conidial conjugation in the Ustilaginales also favor vegetative fusions in the Ascomycetes (*Leptosphaeria Coniothyrium*, *Monilia fructigena*, *Monilia cinerea*), implying that since the two types of fusions are governed by the same physiological conditions they are of a similar nature. In this connection, Gwynne-Vaughn's (1930) nutritive heterothallism which she has described in *Humaria granulata* and which will be discussed more fully presently, should be mentioned. Finally, Kniep's own evidence for multiple fusions of conidia (1926) raises further doubts as to the nature of the process.

It is certainly necessary to recognize the possibility of haploid infections in the smuts (by sporidia of one sexual type) which Zillig (1921) and Goldschmidt (1928) claim do not take place in the anther smut, and which

Dickinson (1927) claims is impossible for *Ustilago levis*, as he finds such germ tubes cannot penetrate the host tissue. The behavior of Blizzard's uninucleate mycelium in *Urocystis Cepulae*, the condition in the corn smut *Ustilago Zeae* where conidial fusions have never been obtained with any degree of certainty in culture, and Boss's uninucleate chlamydospores of *U. Ischaemi*, all throw doubt upon the assumption of the necessity of sporidial fusions and the diploid condition for initiating the parasitic mycelium. In the corn smut, *U. Zeae*, clamp connections between sexually different mycelia have been reported (Seyfert, 1927; accepted by Stakman, 1927) as originating the binucleate condition, but Seyfert's evidence that these are clamp connections, and not ordinary branches is far from convincing. Hanna's (1929) description of fusion of germ tubes in the host tissue, arising from sexually different sporidia in *U. Zeae* has already been mentioned, but the admission is made in both these cases that the haploid mycelium can penetrate the host, although Hanna denies that it is able to produce galls. Eddins's recently reported case (1929) of gall production in *U. Zeae* when a monosporidial culture was used in inoculation is interpreted by him as evidence for homothallism. Parthenogenetic or androgenetic development is the only interpretation that can be made if the sex chromosome theory of sex determination at reduction division is accepted, and the conidia are regarded as gametes, unless an abnormal case is postulated in which reduction has failed to take place, thus giving rise to single conidia with both sex tendencies. Although it is necessary to know the cytological data before a decision can be reached in this case, it is very likely that we have here still another example of the fungus going through its development in the haploid condition.

Much of the evidence on the lack of pathogenicity of haploid conidia is based on cultures grown for shorter or longer periods on artificial media, as were Stakman's sexual strains, and it is quite possible that ordinary loss of virulence so often seen in bacterial cultures, may have more to do with this lack of pathogenicity than absence of the opposite sexual strain. If it is proved that the unconjugated as well as the conjugated conidia have the power to successfully infect the host, then the evidence that the conidium is a cell that has reached maturity, i.e. that it has become different from an ordinary vegetative cell, is gone, and it is not possible to consider it a gamete.

The question of heterothallism-homothallism in the smuts cannot be decided solely by infection experiments even when monosporidial cultures are used. Eddins's case is an example of this. The cytological data must be accumulated for the various species studied. It is this lack of cytological evidence that is the weak point underlying all Kniep's reasoning in relation

to the smuts. We have very little evidence of what the nuclear condition is in the conidium when it sends its germ tube into the host, and not much more as to the nuclear condition of the mycelium in its earliest stages in the host plant. Such a condition as that of mine (fig. 1, plate 27) where the infecting hypha apparently arises directly from a spore brings to mind the smuts that do not form conidia, but infect directly from the spore (*Ustilago Tritici*, *U. nuda*). It is necessary to determine the number of nuclei in the hyphal cells of the smut fungus in the early stages of infection for a great number of different smuts before the sex relations in the group can be determined. A study of these early stages, similar to the cytological study which Sass (1929) has made on certain two-spored forms of agarics would be of great value, but the danger must be guarded against of correlating too closely our ideas of heterothallism and homothallism and gametophyte and sporophyte with a so-called haploid or diploid nuclear condition. The unusual case (fig. 1) of an infecting hypha proceeding directly from a spore without the formation of promycelium and conidia brings to mind the formation of the embryo sac in *Lilium*, where the reduction divisions take place in the germination of the embryo sac instead of in the formation of the megaspores. The sac, although containing a diploid nucleus at first, and later the differentiated spore nuclei, is nevertheless to be regarded as belonging to the gametophytic, which is generally the haploid, generation, although it is unquestionably at first a single cell containing the full complement of sporophyte nuclear material. An infecting hypha from a smut spore is, therefore, to be considered as gametophytic and equivalent to the binucleate infecting germ tubes which arise from single conidia. In order to establish the diplophase, paired nuclei should divide, as in the formation of aeciospores in the rusts.

That other factors besides those involving a differentiation into sexual strains may occur, seems largely to have been lost sight of by the recent investigators of the smuts. This point is stressed especially in a recent paper by Gwynne-Vaughn and Williamson (1930) who find in the Ascomycete *Humaria granulata* that although all thalli are monoecious or female (bear oogonia), nevertheless it is only when mycelia from different kinds of spores (plus and minus) are brought together that the further development of the archicarp into a fruit body takes place. This is described as nutritive heterothallism, and the implication is that some substance is transmitted from the plus to the minus mycelium or vice versa which is specific for the further development of the archicarp. Cayley (1923) reported a type of heterothallism for *Diaporthe* in which unlike strains exhibited an aversion (presumably a toxic action) for each other, which hinders the growth of either colony in the area common to both of them as they approach sufficiently close to each other.

This is directly opposed to evidence presented by Dodge for *Ascobolus magnificus*, who observed this antagonistic tendency between like strains when grown in culture, and is also opposed to the evidence of Brunswik, who reports in *Coprinus Friesii*, *C. fimetarius*, *C. ephemerus*, a distinct separation, often in the form of a line or groove, appearing between the aerial mycelium of the haplonts which did not unite and form clamp connections, and which are therefore considered to be alike.

In explanation of the condition of heterothallism in the fungi, there have appeared in the literature three principal hypotheses:

(1) That it is a sexual relation, that a condition exists of dioecism in the gametophyte whereby sexually differentiated strains exist side by side. This view seems probable in such cases as Dodge's strains of *Caeoma nitens* which produce uninucleate aecidiospores with or without spermogonia, and Narasimhan's male and female strains of *Phytophthora* in which the male strains are reported as producing the antheridia and the female strains the oogonia. The dioecism is therefore structurally expressed. In the Basidiomycetes, as Gwynne-Vaughn points out, in the absence of sexual organs, there is no evidence of specific sex differentiation. In the Zygomycetes, recent researches of Satina and Blakeslee (1929) indicate that, contrary to Blakeslee's earlier findings (1915), there is here likewise no structurally expressed dioecism.

(2) That it is a nutritive condition, as Gwynne-Vaughn suggests, in which some substance is transmitted from the plus to the minus mycelium or vice versa when the mycelia meet and anastomose, which favors reproduction.

(3) That it is a condition of antagonism or aversion as described by Cayley, but which is not limited to unlike strains, as she maintains, but is also found among strains which by the criteria of pairing are regarded as alike (Dodge and Brunswik). There are then two fundamentally different viewpoints underlying this phenomenon of aversion. From Cayley's standpoint, it is the characteristic of aversion suddenly appearing in a strain that marks it as different from its parent, and this difference finds its ultimate expression in the production of perithecia, whereas the parent strain formed only pycnidia.

The evidence of complete fertility among different geographic races (Hanna, 1925), and the spontaneous change from heterothallism to homothallism are serious difficulties in explaining heterothallism purely from the standpoint of sex differences. Moreover, as Cayley (1930) points out, to explain heterothallism from the standpoint of nutrition necessitates an explanation of why only half the combinations are fertile. The evidence seems to favor the view that heterothallism is due to incompatibilities aris-

ing in the development of the individual, rather than to a genetically predetermined constitution. The widespread occurrence of incompatibilities in the reproduction of the higher plants (Stout) suggests that such conditions are probably also operative in the reproduction of the fungi.

The condition in *Ustilago Avenae*, in which both a modified type of flower infection is claimed by some workers (Zade and his students, and Gage), and successful seedling infection is demonstrated without question by others (Reed, Faris), opens up the question whether different physiologic forms may not infect their hosts in different ways. Tisdale and Griffith (1927) suggested this in connection with their experiments with *U. nuda*, but in their case another condition complicates the situation. They secured seedling infection with this flower infecting smut after removing the hulls from the seed, a procedure which in barley often results in injury to the seedling so that the question is raised whether they may not have secured infection by first wounding the embryo. This question of wounding is also brought up in relation to the use of the hypodermic needle in inoculation. Faris and Reed (1925) secured a localized infection of sorghum by injecting spores of *Sphacelotheca cruenta*, considered a seedling infecting smut, into the boot, growing point, or between the leaf sheath and internode. The usual type of infection is a general systemic infection through seedlings, although their investigations also indicate the existence of a local type occurring in nature. Griffith (1928) found that by using the hypodermic needle in inoculating corn with *Ustilago Zeae* she was able to secure infection in otherwise resistant varieties. These results of Griffith indicate that the corn smut, and possibly other smuts also, may be wound parasites. There is, however, nothing to indicate that in spite of this apparent breaking down of distinctions between the various physiologic forms by first wounding the host, there may not also be varied modes of infection in nature, so that some of the physiologic forms infect more easily by one method, others by another method. Zehner and Humphrey (1929) however, were not able to infect the resistant Nakano Wase barley by injection with *U. nuda*. That there are varied modes of infection of sorghums by *Sphacelotheca cruenta* has already been mentioned. Besides general systemic infection through seedlings, Faris and Reed also found local types of infection, and a type of shoot infection. Some such explanation as this might account for the results obtained with *U. Avenae* by Gage's flower inoculation, and by Reed's dry spore dusting method of seed inoculation, and especially Zade's and Gage's lack of success with the dry spore dusting method. However, it must not be overlooked that other factors may have contributed to this lack of success. Reed and Faris (1924) have shown the importance of environal factors at the time of germination upon percent-

age of infection, the temperature during the period of germination and the percentage of moisture in the soil being especially important in its effect on the resulting percentage of smut. Different methods of inoculation with *U. Avenae* have recently been tested and compared by Von Rosenstiel (1929) who finds Reed's method most effective.

The criteria for physiologic forms as distinguished by Reed and Faris are inherent differences in infecting power between spores of different strains of smut on a wide variety of hosts. Stakman, on the other hand, suggests that there is complete lack of infecting power in some forms and this indicates that certain physiologic forms are made up of sporidia entirely of one sex.[4] It seems hardly possible that lack of the power to infect a certain host in the forms studied by Reed and Faris is due to absence of one strain of a heterothallic pair, since both sexual strains should be present when an abundance of dry spores are used as inoculum. The question of heterothallism, therefore, does not enter into a consideration of the physiologic forms described by Reed and Faris, nor in the form of *U. Avenae* (Reed's Missouri strain) used in this paper. Stakman's physiologic forms, among which he names three from different localities (New Hampshire, Mississippi, and Canada) are perhaps isolated sexual strains, and they are so interpreted by the investigators themselves. Still these strains were presumably obtained in the spore form from the three localities mentioned, and since monosporidial cultures are not indicated, we are left to conjecture on how the separation of the sexual strains took place. It is possible that dioecism of the gametophyte or the parthenogenetic or androgenetic development of gametes may give cultures which would in time become so specialized as to be true physiologic forms. An original separation of sexual strains may have eventually resulted in the ability of the fungus to complete its entire life cycle on one specific host independently of the other strain, and the inability to infect other hosts unless invigorated by the opposite strain. A suggestion of this separation of the sexes and their isolation on different hosts is indicated in Narasimhan's (1930) recently reported study of *Phytophthora* on *Areca*, *Santalum*, *Loranthus*, and *Jatropha*. The strains on *Areca* and *Loranthus* are said to produce the antheridia, those on *Santalum* and *Jatropha* to produce oogonia, when grown in com-

[4] In a more recent publication (Mutation and hybridization in *Ustilago Zeae*. Univ. Minn. Agr. Sta. Tech. Bull. *65:* 1–108. 1929) which has just come to my notice, Stakman and Christensen appear to change their viewpoint, and offer evidence in support of the pathogenicity of some unisexual or monosporidial lines. They state that pathogenicity is not a function of sex only. Pathogenicity and sex are to a considerable extent interdependent in *Ustilago Zeae*, but 'it is not known to what extent pathogenicity is a function of sex factors, and to what extent of physiologic factors.'

bination. The former are considered male strains and the latter female strains. Each strain is capable of maintaining itself by asexual reproduction on its own specific host. The oospore then is an evidence of sexual reproduction, as can be shown by bringing together two dioecious gametophytic strains. But even in this case of Narasimhan's, absolute proof of an antheridia-bearing and an oogonia-bearing strain must be forthcoming before the dioecism of the gametophyte is definitely established.

In the systemic smuts, on the other hand, since we have no evidence of the presence of the fungus until the resting spore is produced, it is necessary to study the method of origin of the spore before a decision can be reached as to whether a given physiologic form has two sexual strains or is an isolated strain of one sex. There is in the smuts no possibility of differentiating a male or female strain, as is possible in the short cycled *Caeoma nitens* strains discovered by Dodge, as respectively with or without spermogonia. It may be that in some such way as that suggested in *Phytophthora*, many of our grain inhabiting fungus parasites have become specialized on their respective hosts. The only trace of sexuality which remains is a dioecism which is only apparent in certain cases, as in those described by Stakman in which the strains have become separated from their specific hosts, and which is masked in other cases by automictic development or further parthenogenetic or androgenetic development.

The relation of host and pathogen in these specialized smuts is of great interest. In the resistant Black Mesdag oat, the smut fungus is unable to get anywhere near the growing point, where in the case of a susceptible variety like Victor, the parasite first becomes actually established inside the host. Fixed material of Black Mesdag seedlings seven, ten, and fifteen days old, showed only very infrequently mycelium in the tissues of the coleoptile, and then up near the coleoptile tip, although examination of fresh material showed mycelium constantly present in the inner epidermis of the coleoptile. It seems clear that this mycelium, seen in the living cells of the inner epidermis, when strips were peeled off, was growing lengthwise of the coleoptile, but was not making its way across the space between the coleoptile and the first leaf, nor had made its way through the tissues of the coleoptile. How it entered is a question that I have not yet decided, but its apparent inability to penetrate the outer epidermal cells might suggest the small slit on the side of the coleoptile away from the scutellum through which the leaves emerge. Percival (1921) describes and figures this opening (figs. 9, 35) in the wheat plant. In none of these seedlings was there any trace of mycelium in the leaves, leaf primordia, or growing point. Resistance, therefore, in Black Mesdag seems to be due to a lack of ability on the part of the fungus to make its way to the growing point of the stem.

In the rusts, however, as reported by Allen, resistance constitutes a hypersensitivity of the host cell to the fungus. The host cell is killed outright in many cases at the first attempt of the fungus to establish haustoria, and it is this incompatibility between host and pathogen that prevents the development of the rust. Susceptible varieties, as studied by Allen and also by Rice, show compatibility between host and pathogen in the rusts, since the host cell is not killed, but to all appearances functions normally, at least at first. In the susceptible variety of oats, Victor, compatibility between host and pathogen reaches such a high degree of development that the fungus causes no change in the appearance of the cell. In cells of the coleoptile and mesocotyl the nuclei, more often than not, are well stained, showing nucleoli, the cytoplasm of the cell is easily distinguished, and there is little difference between cells invaded by hyphae and those not. The pathogen, on the other hand, shows various appearances—the empty hyphae above referred to, long drawn-out thread-like hyphae, and swollen hyphae very similar to the hyphae described by the older authors as encased in cellulose sheaths. These phenomena I interpret rather as degeneration of empty hyphal walls than as indicating a mutualistic host-parasite relation. In the cells of the growing point, the large host nuclei typical of embryonic regions stain well. The hyphae lie in short plump fragments between these cells.

Roesch has recently indicated that empty hyphae are more frequently found in a resistant host (*Avena brevis*) than in a susceptible one, and suggests that they are effects of the host relations with the parasite. I, however, find these empty hyphae so frequently in my material from an extremely susceptible host, that I think they are a normal stage in penetration. The fungus seems to reach to the growing point before developing a permanent mycelium. Its protoplasm remains in the hyphal tip and the hyphal wall remains as an empty tube which may collapse.

There is apparently a much more rapid development of mycelium in the young oat seedling infected with *Ustilago Avenae* than in the young wheat seedling infected with *Tilletia Tritici* as described by Woolman (1930). The seedlings studied by him were much older than my seedlings, since the age recorded as six or seven days actually referred to the length of time after emergence of the seedling above the ground, and such emergence in some cases was as long as ten days following sowing. In my work, on the other hand, the age of seedlings has been reckoned as from the day of planting. In these seedlings, however, the bunt fungus had apparently not penetrated into the host plant as far as I have diagrammed for *U. Avenae* in seedlings four or five days after sowing. Actual invasion of the growing point is not mentioned by Woolman in plants younger than sixty days old.

In relation to the mycelium in the growing region, I do not find a strictly intercellular mycelium as maintained by Woolman for his third phase of infection, except in the cone of the growing point. Below this, as I have already described on page 473, I find both intercellular and intracellular mycelium.

SUMMARY

1. The dry spore dusting method of inoculation in loose smut of oats (*Ustilago Avenae*) proved highly successful, giving 100 per cent infection (110 plants, in 4 plantings) in my experiments.

2. The distribution of mycelium of *Ustilago Avenae* was studied in oat seedlings inoculated by means of this dry spore dusting method, and was found to be as follows:

(a) In seedlings from 1 to 4 days old, mycelium was found in the coleoptile from its tip to the coleoptile node, in the space between the first leaf and the coleoptile, in the first leaf, in the tissues of the node, and in the mesocotyl in the vicinity of the coleoptile node (figs. 1–7).

(b) Germ tubes penetrating the epidermis of the coleoptile (initial penetration) showing the characteristic 'holes' in the cuticle at the point of penetration noted by Brefeld, were observed in a three day old seedling. In one case a chlamydospore outside the seedling was found still attached to a germ tube which had made its way into and across the epidermal cell.

(c) No mycelium was found in the scutellum, the root node, or the lower portion of the mesocotyl in these 1–4 day old seedlings.

(d) In seedlings 5–8 days old, the mycelium was found distributed throughout the tissues of the coleoptile and mesocotyl (figs. 10–19).

(e) In older seedlings (13–30 days old), the growing region from the coleoptile node up to the cone of the growing point showed mycelium in the closely crowded nodes. This mycelium was especially abundant down near the coleoptile node, but became more and more infrequent up near the point of origin of the youngest leaf primordium (figs. 9, 20). Although mycelium was observed in the vicinity of these very young leaf primordia, none was found in the cone of the growing point.

(f) In seedlings over a month old (31, 38, 40 days old), the mycelium was found in the apex of the cone of the growing point (text fig. 21).

3. A cytological study of the mycelium in the seedling shows that:

(a) Intracellular mycelium is abundant in all tissues of the coleoptile, the mesocotyl, the coleoptile node, and in the internode between this and the first leaf. These hyphae are frequently long and show signs of degeneration (collapsed and empty portions). Intercellular mycelium is also present.

(b) As the mycelium makes its way up through the growing region

(region of closely crowded nodes), it becomes increasingly difficult to make out whether it is intracellular or intercellular. The hyphae appear shorter here, and there is evidence of their being both intracellular and intercellular.

(c) In the tip of the growing point, the mycelium is intercellular.

(d) There is a characteristic widening of the hyphae at the point where a host cell wall is penetrated, giving a somewhat funnel-shaped appearance to the hypha.

(e) Unusual appearances in the hyphae, such as empty portions, collapsed portions, reduction of the hypha to a filiform strand, and hyphae with thickened or gelatinized walls, illustrated in my figures 13, 16–21 (plates 29, 30) I regard as normal phases in the development of the hyphae as they grow toward the apical meristem.

(f) The number of nuclei in the hyphae vary. Some portions appeared uninucleate, others binucleate, and others multinucleate.

4. A preliminary study of Black Mesdag oats, a resistant variety, has shown the mycelium usually in the cells of the inner epidermis of the coleoptile, but only very rarely anywhere else in the coleoptile.

This work was completed in the botanical laboratory of Columbia University under the direction of Professor R. A. Harper, to whom I am indebted for valuable suggestions, criticisms, unfailing interest, and inspiration. I also wish to acknowledge my indebtedness to Dr. George M. Reed of the Brooklyn Botanic Garden for material, valuable suggestions, and greenhouse facilities.

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Explanation of plates 27–30

All figures were drawn with the aid of a camera lucida from sections 10–15 microns thick (except fig. 23) stained with Flemming's triple stain. A Leitz microscope was used with oil immersion objectives 1/16 in. and 1/12 in. and a number 3 ocular, except for fig. 17, where a no. 1 ocular was used. Magnifications range from approximately 633 to 1200 diameters, as indicated in the descriptions.

Plate 27

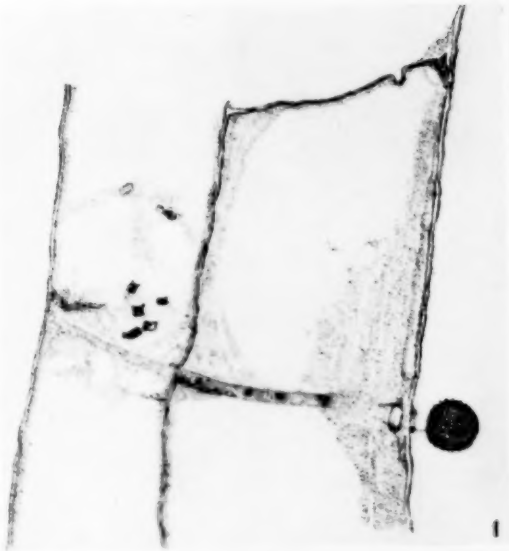
Fig. 1. Promycelium from a spore outside the cuticle, functioning as a germ tube and making its way across an epidermal cell right above the coleoptile node of the three days old seedling diagrammed in text fig. 2. Longitudinal section. ×1200.

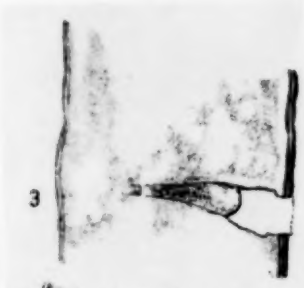
Figs. 2–4. Germ tubes penetrating the epidermal cells of the coleoptile of the three days old seedling diagrammed in text figs. 2 and 3. The holes in the epidermis are prominent. Long. sect. ×1200.

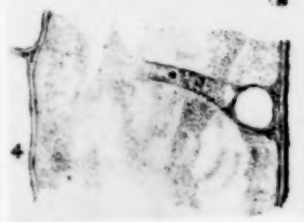
Fig. 5. Degenerating infecting germ tube up near the tip of the coleoptile of the two and a half days old seedling diagrammed in text fig. 5. The hole in the cuticle is distinct. Long. sect. ×1200.

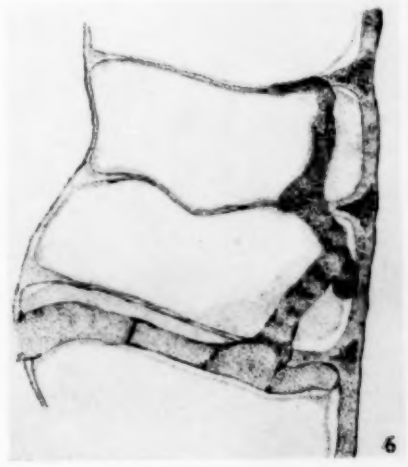
Fig. 6. Hypha in the epidermal cells near the coleoptile node of a seedling five days old cut in cross section. The path of the hypha through the primordial utricle of the cells is striking. ×1200.

Fig. 11. Penetration of cortical parenchyma cells of the mesocotyl of a seedling 15 days old, cut in cross section. A hyphal branch is making its way into an intercellular space; another has formed within the cell. ×1200.









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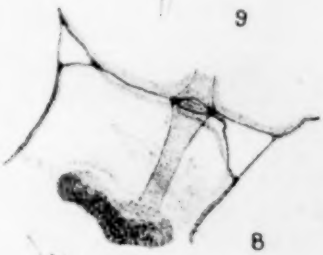
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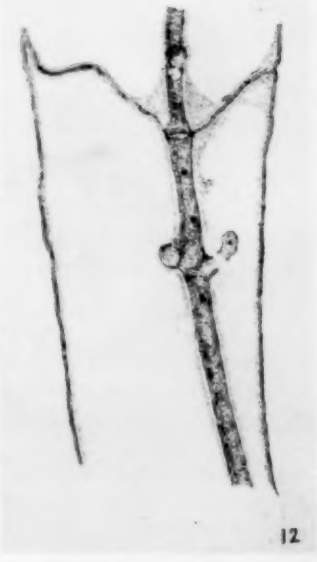
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PLATE 28

Fig. 7. Hypha in the parenchyma cells of the mesocotyl of the same five days old seedling as in plate 27, fig. 6. Beginning penetration of the cell wall is indicated by the minute spot of reddish stain at the point of contact of the hyphal tip with the cell wall. ×1200.

Fig. 8. Penetration through the wall of a cortical parenchyma cell near the central stele of the mesocotyl of a fifteen days old seedling cut in cross section. Broadening of the hypha at the point where it passes through the cell wall is characteristic, as are the deposits of reddish stain on either side of the hypha. ×1200.

Fig. 9. Characteristic funnel-shaped hypha as it passes through host cell wall. Cross section through parenchyma cells of the coleoptile of an eight days old seedling. ×1200.

Fig. 10. Hypha penetrating the cell wall of a cortical parenchyma cell of the mesocotyl of a seedling eight days old, and making its way directly to the nucleus. Cross section. ×1075.

Fig. 12. Longitudinal section through the mesocotyl of a seedling 13 days old, showing a hypha penetrating the cell wall between two cortical parenchyma cells. An intracellular branch has formed. ×1000.

Fig. 14. Longitudinal section through the mesocotyl of a 13 day old seedling showing dense mass of cytoplasm around the tip of a hypha in one of the cortical parenchyma cells. ×1000.

PLATE 29

Fig. 13. Cross section through the cortical parenchyma of the mesocotyl of a seedling eight days old, showing difference in staining capacity and in form of portions of the same hypha. ×1075.

Fig. 15. Longitudinal section through the cortical parenchyma of the mesocotyl of a seedling 13 days old showing mass of host cell cytoplasm around the tip of a hypha. ×1200.

Fig. 16. Cross section through the cortical parenchyma cells of the mesocotyl of a seedling eight days old showing a cytoplasmic mass around the hypha. ×1075.

Fig. 17. Cross section through the cortical parenchyma cells of the mesocotyl of a seedling five days old showing degeneration in portions of a single long hypha (174.60 microns) making its way through five host cells. ×633.

Fig. 20. Longitudinal section through the cells of the cortical parenchyma of the mesocotyl of a seedling twenty days old, showing swollen portions of degenerate appearing hyphae. ×1000.

PLATE 30

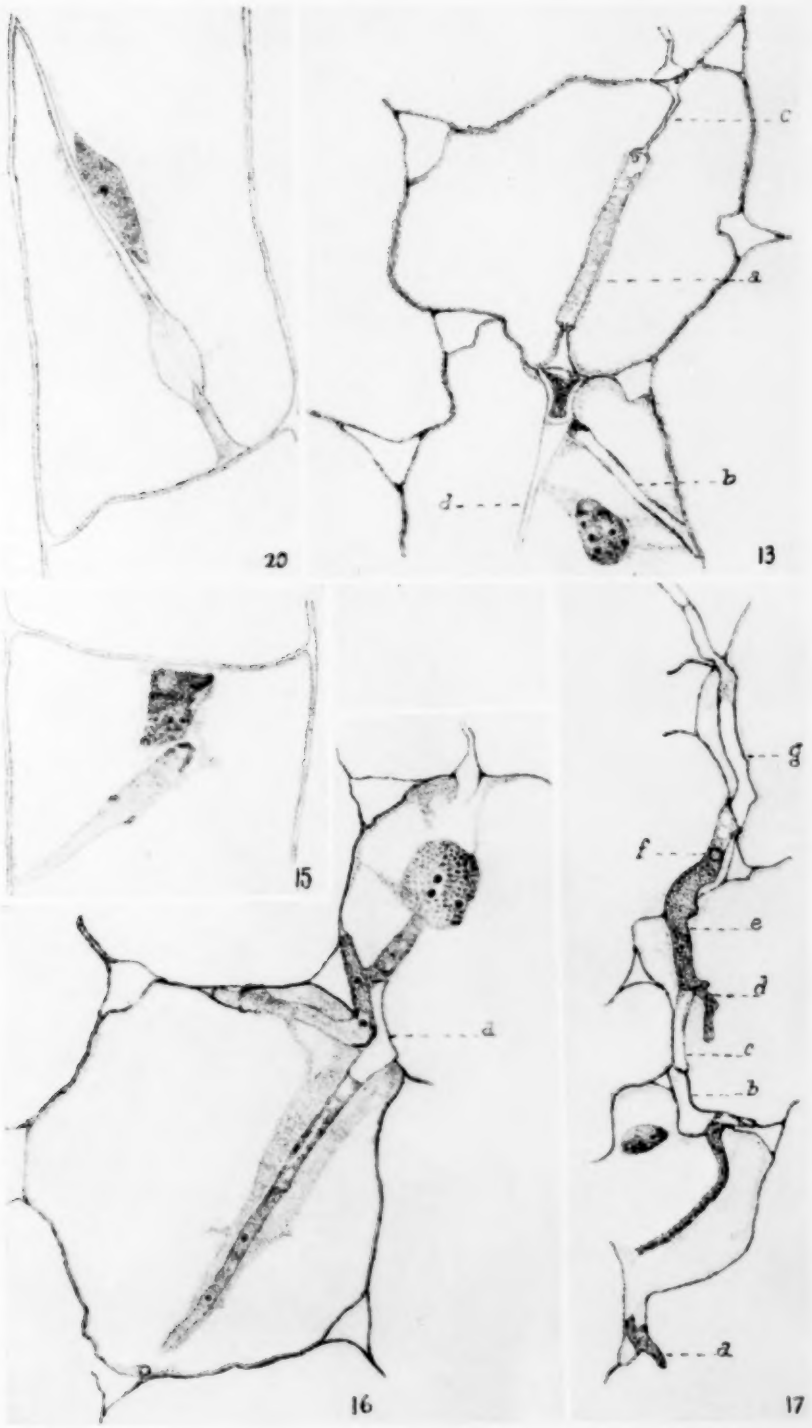
Fig. 18. Longitudinal section through the coleoptile of a four days old seedling showing a tangential view of the cells of the inner epidermis. A hypha, the greater part of which has degenerated to an empty thread, is making its way across these cells. ×1175.

Fig. 19. Cross section at the base of the coleoptile node of an eight days old seedling, showing penetration of parenchyma cells by a hypha which appears to be drawn out in a fine thread, as if stretched. ×1075.

Fig. 21. Same as fig. 20 (plate 29).

Fig. 22. Hyphae in the space between the coleoptile and the first leaf of a seedling two and a half days old, cut in longitudinal section. ×1200.

Fig. 23. Median longitudinal section through a seedling thirty-eight days old, showing an intercellular hypha in the apex of the cone of the growing point. ×1200.



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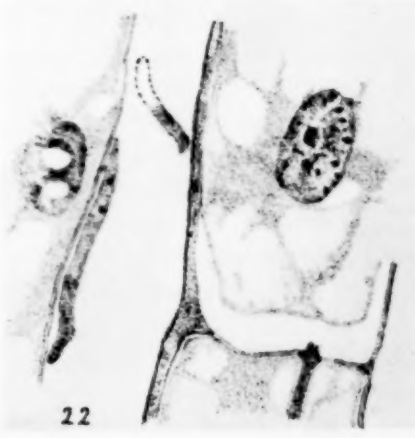

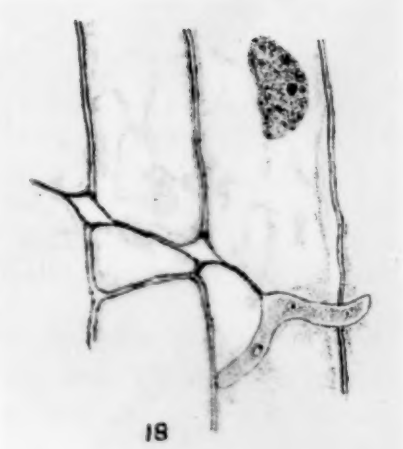

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Plants observed on an excursion to Grand Isle, Louisiana

CLAIR A. BROWN

For the past few years it has been the custom for members of the faculty and students of the Zoology Department of Louisiana State University to make a field trip to study the marine fauna around Grand Isle, Louisiana. The Zoology Department planned to establish a permanent Biological Summer Camp there, and for this reason the writer accompanied the Zoology group on their trip to the island in April 1928, to collect the plants found there.

Grand Isle is located about fifty miles south of New Orleans, Louisiana, at the mouth of Barataria Bay. The island is reached by boat which leaves from Harvey, Louisiana, just across the Mississippi River from New Orleans. The little boat takes about eight hours to make the trip, passes through Harvey's Canal into Bayou Barataria to the town of Lafitte, named after Louisiana's notorious pirate. From here it passes through Bayou Dupont, into the new Government Canal, then through Bayou Cutler, into Bayou St. Dennis, and crosses Barataria Bay to Grand Isle.

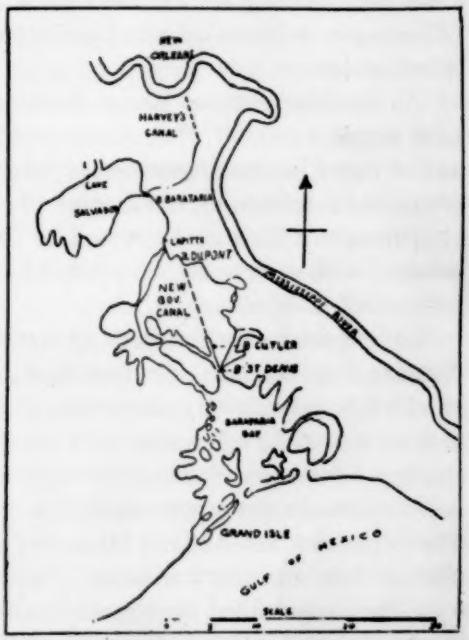

Fig. 1. Sketch map of Grand Isle region.

For most of the way the banks of the canals and the bayous are close enough so that one can identify, at least to the genus, many of the plants seen. At this time of the year many plants were not in bloom and were therefore often indeterminable. The total number of plants recognized and recorded from Harvey's Canal to Barataria Bay can be distributed into sixty-six genera, representing about forty families.

Along Harvey's canal some of the more conspicuous plants are the common weeds which are very abundant throughout Louisiana. Some of those which spring up in the places where man has disturbed the natural vegetation are: sow thistle (*Sonchus asper*), butter weed (*Senecio lobatus*),

spiny thistle (*Carduus spinosissimus*), fleabane (*Erigeron sp.*), vervain (*Verbena* sp.), yellow sweet clover (*Melilotus indica*), false dandelion (*Sitilias caroliniana*), and blackberries (*Rubus sp.*). Here and there one could see the skeletons of last year's plants with new growth coming up around the old dead stalks. The most common of these were Yankee weed (*Eupatorium capillifolium*), coffee weed (*Sesban macrocarpa*), giant ragweed (*Ambrosia trifida*), and the tall dried panicles of the plume grass (*Erianthus sp.*). The principal trees were willows (*Salix* sp.), red maples (*Acer rubrum* and varieties), water ash (*Fraxinus caroliniana*), tupelo (*Nyssa aquatica*) cypress (*Taxodium distichum*), honey locust (*Gleditsia triacanthos*), many of which were festooned with Spanish moss (*Dendropogon usneoides*), and overrun by such vines as *Brunnichia cirrhosa* Banks, pepper vine (*Ampelopsis arborea*), and wild grapes (*Vitis sp.*). At the edge of the banks of the canal and floating in the water were cat-tails (*Typha sp.*), water millet (*Zizaniopsis miliacea*), water hyacinth (*Piaropus crassipes*), and species of *Alternanthera*.

As the boat entered Bayou Barataria, where man's destructive influence was less evident, the most conspicuous vegetation was the trees such as live oak (*Quercus virginiana*), cypress (*Taxodium distichum*), water ash (*Fraxinus caroliniana*), red maples (*Acer rubrum*), and sweet gale (*Myrica sp.*); most of which were covered by the drooping gray Spanish moss and overrun with vines. As we neared Lafitte, we began to see more of the palmetto (*Sabal minor*).

Lafitte marks the southern extremity of the auto road. Here, a short distance from the town, the firm land gives away to a low marsh, the level of which is only slightly above that of the new Government Canal. From here we passed for miles through a marshy region called 'prairies' composed chiefly of reed grass (*Phragmities communis*), water millet (*Zizaniopsis miliacea*), and a few scattered clumps of the groundsel tree (*Baccharis sp.*). These 'prairies' extended as far as one could see on either side of the boat, marked here and there with an 'island' on which the conspicuous plants were the moss-draped cypress and palmettos. On entering Bayou Cutler, the boat passed into marsh lands, lower than the preceding, which appear to be affected by the tides, and which are called on the United States Geodetic Survey Charts, 'trembling prairies.' The plants throughout this region were lower in height than those in the previously mentioned marsh, and the dominant plants were grasses, rushes, and sedges, among which were salt meadow grass (*Spartina patens*), with scattered plants of *Salicornia* and *Baccharis*. It was a monotonous appearing region but would be certain to yield a good many interesting plants if one could stop and explore the marsh.

As the boat entered Barataria Bay the shores soon faded into long thin lines against the horizon. One of the stops which the boat made was at Cabinash, Louisiana, a little shrimp-fishing settlement. This village consists of a cluster of houses and a store, all built upon piles on a bar which protrudes about a foot above the surface of the water. The dominant vegetation here was composed of rushes, sedges, and a few other plants, which were not recognized.

At last, after a considerable piece of open water, Grand Isle came into view, seen at first as a low-lying smudge against the horizon, which, as we drew nearer, began to take on a definite shape. Grand Isle is unique in one respect: it has one of the few sandy beaches on the Gulf coast of Louisiana. The island lies with its long axis approximately North-East and South-West, with a length of about six miles and it varies from one-half to a mile in width. In bygone days the island was famous as a summer resort; a place to escape the terrific heat of the city in midsummer.

Lafcadio Hearn[1] has described the grim tragedy that overtook Grand Isle, and especially Grand Terre, when the waters of the gulf were driven over the islands by the hurricane of August 10, 1879. The interesting thing he described was the dikes which had been constructed to keep the sea off the sugar cane lands. It is evident from his account that what is now the fine shallow bathing beach is all that is left of the sugar cane fields.

Of the botanists who have visited the island, Americus Featherman[2] has left us perhaps the best record of the plants found there. It is, however, but a meager list of twenty species. He described two new species from the island, although they were not considered valid species by Asa Gray.[3]

At this point the writer will take up his own collections and studies made during the three days of his stay. The three major plant associations found here may be correlated with three distinct observed differences in the composition of the soil. The first of these is the association which comprises the gulf side of the island and varies in width. This association can in turn be divided into two minor sections, the one characterized by drifting sand, which contains a few scattered plants such as *Ipomoea Pes-Caprae* (L.) Sweet, *Euphorbia* sp., and *Hydrocotyle* sp.; and the other characterized by a thin but compact turf, over which sand may drift in small amounts. Among the grasses and sedges here were *Centella repanda* (Pers.) Small and *Dichromena colorata* (L.) Hitchcock. The following is a list of plants collected in this association:[4]

[1] HEARN, L. Chita: a memory of Lost Island. Harper's Mag. **76**: 733–767. 1888.

[2] FEATHERMAN, A. Report of the Botanical Survey of Southern and Central Louisiana. Legislative Documents of Louisiana, pp. 1–132. 1871.

[3] GRAY, A. Amer. Jour. Sci. III. **2**: 374–375. 1871.

[4] Only specimens in flower or fruit were collected.

Bacopa Monniera (L.) Wettst.
Cenchrus pauciflorus Benth.
Centella repanda (Pers.) Small
Centunculus minimus L.
Chaetochloa purpurascens (H.B.K.) Scribn. & Merr. (?)
Chloris petraea Swartz
Dichromena colorata (L.) Hitch.
Eleocharis albida Torr. (?)
Eleocharis sp.
Eragrostis secundiflora Presl.
Erigeron repens Gray
Euphorbia sp.
Festuca octoflora Walt.
Hydrocotyle sp.
Ipomoea Pes-Caprae (L.) Sweet
Isnardia palustris L.
Kyllinga brevifolia L.
Oenothera laciniata Hill
Panicum repens L.
Panicum sphaerocarpon Ell.
Paspalum ciliatifolium Michx.
Plantago virginica L.
Phyla lanceolata (Michx.) Greene
Rubus trivialis Michx.
Ruppia maritima L.
Salsola Kali L.
Serinia oppositifolia (Raf.) Kuntze
Sisyrinchium sp.
Tissa marina (L.) Britton
Yucca aloifolia L. (?)

The second major association is located in the center of the island. The soil is sandy with a much larger amount of black organic matter present as compared with the turf area of the first association. Trees and shrubs form the dominant native vegetation. The live oaks are typical of this area and those nearest the seabeach are bent in the direction of the prevailing winds. Oleanders line the little lanes which run cross-wise of the island and here and there other shrubs and trees have been planted. It is in this area that the inhabitants build their cottages and plant the prolific truck crops of snap beans and cucumbers. The following list is of the plants collected in this association:

Alsine aquatica (L.) Britton
Alsine media L.
Ampelopsis arborea (L.) Rusby
Apium Ammi (L.) Urban.
Arabis virginica (L.) Trelease
Berchemia scandens (Hill) Trelease
Buchnera elongata Sw.
Callitriche sp.
Capriola Dactylon (L.) Kuntze
Celtis laevigata Willd.
Cerastium viscosum L.
Chaerophyllum Tainturieri Hook.
Chelidonium majus L.
Chenopodium anthelminticum L.
Commelina erecta L. (?)
Cyperus esculentus L.
Dichondra carolinensis Michx.
Diospyros virginiana L.
Fagara Clava-Herculis (L.) Small
Galium Aparine L.
Galium Claytonii Michx.
Geranium carolinianum L.
Gleditsia triacanthos L.
Gnaphalium purpureum L.
Gymnostyles anthemifolia A. Juss.
Ilex vomitoria Ait.
Juncus aristulatus Michx.
Juncus brachycarpus Engelm. (?)
Lantana horrida H.B.K.
Medicago arabica All.
Melia Azedarach L.
Morella cerifera (L.) Small (?)
Morus alba L.
Nerium Oleander L.
Oenothera laciniata Hill
Panicum lanuginosum Ell.
Panicum sphaerocarpon Ell.
Paspalum Larranagi Arech.

PHLOX DRUMMONDII Hook.
POA ANNUA L.
QUERCUS VIRGINIANA L.
RANUNCULUS MURICATUS L.
RANUNCULUS SCELERATUS L.
RHUS RADICANS L.
RUBUS TRIVIALIS Michx.
RUMEX LANGLOISII Small (?)
SAGINA DECUMBENS (Ell.) T. & G.
SALIX sp.
SAMBUCUS CANADENSIS L.
SCIRPUS CARINATUS (H. & A.) Gray
SIDA sp.
SITILIAS CAROLINIANA (Walt.) Raf.
SMILAX BONA-NOX L.
SOLANUM CAROLINENSE L.
SOLANUM NIGRUM L.
SONCHUS OLERACEUS L.
SPECULARIA PERFOLIATA (L.) A. DC.
SPHENOPHOLIS OBTUSATA (Michx.) Scribn.
SPOROBOLUS INDICUS (L.) R. Br.
SYNTHERISMA SANGUINALE (L.) Dulac.
TAMARIX GALLICA L.
URTICA CHAMAEDRYOIDES Pursh.
VERBENA BONARIENSIS L.
VERBENA XUTHA Lehm.
VERONICA PEREGRINA L.
VICIA LUDOVICIANA Nutt.
VIGNA REPENS (L.) Kuntze
XANTHOXALIS STRICTA (L.) Small

The third major association lies on the north or land side of the island. The soil of this region is composed of a black muck laid down over a shell bottom. The vegetation here is that of a typical salt marsh; low shrubby plants and rushes predominate, then come succulents such as *Salicornia*. Very few plants of this area were in condition to identify and for that reason this list is short. The following species were collected in this association:

APIUM AMMI (L.) Urban.
AVICENNIA NITIDA Jacq.
BACCHARIS sp.
BORRICHIA FRUTESCENS (L.) DC.
CUSCUTA Sp.
JUNCUS ROEMERIANUS Scheele
PERSICARIA sp.
PHYLA LANCEOLATA (Michx.) Greene
PLANTAGO VIRGINICA L.
SALICORNIA AMBIGUA Michx. (?)
SALICORNIA HERBACEA L.
TISSA MARINA (L.) Britton
TYPHA sp.

One set of these plants has been deposited in the Louisiana State University Herbarium, and duplicates of most of them are also in the Herbarium of the University of Michigan.

The writer wishes to acknowledge his indebtedness to Dr. A. S. Hitchcock, who kindly identified most of the grasses.

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BATON ROUGE, LOUISIANA

INDEX TO AMERICAN BOTANICAL LITERATURE
1928–1930

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

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